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# Nicosulfuron tolerance and population dynamics of woolly cupgrass (Eriochloa villosa)

Ming-Chung Liu
*Iowa State University*

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# Nicosulfuron tolerance and population dynamics of woolly cupgrass (Eriochloa villosa)

by

Ming-Chung Liu

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DOCTOR OF PHILOSOPHY

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This is to certify that the Doctoral dissertation of

Ming-Chung Liu

has met the dissertation requirements of Iowa State University

Signature was redacted for privacy.

Major Professor

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For the Graduate College

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# INTRODUCTION

Woolly cupgrass was found to be tolerant to atrazine (2-chloro-4-ethylamino-6-isopropylamino-s-triazine) in early herbicide trials (Strand and Miller 1980). Chemical control of woolly cupgrass in corn (Zea may L.) is highly variable (Owen et al. 1993; Schuh and Harvey 1991). Postemergence nicosulfuron (2-[[[[(4,6-dimethoxy-2-pyrimidinyl)amino] carbonyl]amino]sulfonyl]-*N*,*N*-dimethyl-3-pyridicarboxamide) application usually provides satisfactory control of the main stem of a woolly cupgrass plant. Occasionally, woolly cupgrass will initiate tillers after nicosulfuron application. These plants likely do not compete with the crop nor affect the crop yield, but they are able to produce seeds and recharge the soil seedbank. Larger plants initiate new tillers faster than smaller plants.

There are several hypotheses for tiller emergence following nicosulfuron application. Since woolly cupgrass is able to produce a large number of tillers (Owen 1990), rapid tiller development may contribute to the tiller regrowth or new tiller initiation. If woolly cupgrass had a pattern of tiller development similar to wheat (Triticum aestivum L.) (Klepper et al. 1982) where the coleoptilar tiller initiated from the coleoptilar axil which remained in the seed, it might escape herbicide treatment. Seed reserves could support woolly cupgrass seedling growth for a short period of time. Given that woolly cupgrass has a relatively large seed, the seed reserves might support tiller initiation and growth after main stem was killed by herbicide.

The mechanisms regulating tiller emergence following nicosulfuron application in woolly cupgrass have never been documented. Research was conducted to study the tillering pattern of woolly cupgrass, a microscopic study of tiller development was done, and

herbicide translocation to the newly initiated tiller buds was observed. Experiments were also conducted to determine whether seed reserves played a role in tiller regrowth. Finally, experiments investigating the effect of various herbicide rates and main stem excision on tiller emergence were initiated.

Woolly cupgrass seeds can germinate continuously during the growing season and seedlings from late germination events usually escape herbicide treatment and add to the management problem (Owen 1990). Most herbicide evaluations for woolly cupgrass control emphasize early season control (Owen et al. 1993; Schuh and Harvey 1991). Little information about woolly cupgrass germination responses to tillage is available, and germination response to the residual effects of preemergence herbicides is also lacking. The other objective of this study was to investigate the effects of tillage and preemergence herbicides on woolly cupgrass populations and germination depth. It is hoped that the information obtained from these studies will provide a solid interpretation for tiller regrowth and result in better management of this weed.

**Dissertation organization**

The research is presented as four research papers: the first three papers explore the mechanisms for woolly cupgrass surviving nicosulfuron and the last paper deals with tillage and preemergence herbicide effects on woolly cupgrass population dynamics. A literature review precedes the first paper and a general conclusion follows the last paper. A literature cited section for literature that was cited in the general introduction and literature review follows the general conclusion.

# LITERATURE REVIEW

## Tiller development

Grass seedlings initiate growth of the main stem from the seed. Tiller initiation and growth has been studied in great detail in wheat (Williams et al. 1975). The coleoptile is the sheath enclosing the epicotyl in the embryo of Poaceae; prophyll is a two-keeled structure enclosing the tiller bud and is similar to the coleoptile. Coleoptile and prophyll are sometimes interpreted as the first leaf of the epicotyl and the tiller (Clark and Pohl 1996; Esau 1977). Buds form in the axils of most leaves. Tiller buds become squeezed and flattened by pressure from the tightly wrapped developing leaf bases (Williams et al. 1975). Tiller buds make early vascular connections with the adjacent nodal plexus and bundles descending from higher leaves. Tillers are therefore connected to the entire vascular system of the plant main axis.

Primary tillers originate from the axils of leaves on the main stem, secondary tillers originate from the axils of leaves on primary tillers. An aggressive grass species may produce many orders of tillers by repeating the process of tillering (Langer 1979). Tillering pattern has been widely studied in wheat (Friend 1965; Rawson 1971), barley (Hordeum vulgare) (Cannell 1969a), ryegrass (Lolium rigidum) (Neuteboom and Lantinga 1989), and tall fescue (Festuca arundinacea Schreb.) (Skinner and Nelson 1992, 1994).

Tiller emergence has long been the accepted criterion for the presence of tillers in agronomic studies, and even in physiological studies of apical dominance in the Poaceae. Nevertheless, tiller buds have been growing for days or even weeks before they emerge (Williams et al. 1975).

**Leaf and tiller identification**

Vegetative development in cereal grains includes the formation of leaf primordia, the associated axillary buds and the subsequent development of mature leaves and tillers from these primordia and buds. In order to quantify cereal seedling development, leaf and tiller identification systems are needed. Early systems for describing cereal seedling development were developed by Feekes (Large 1954) and later modified by Zadoks et al. (1974). Feekes used a score of 1 to represent an emerged but untillered plant, a score of 2 represented that tillers were being produced, and 3 indicated the end of tillering. This system had little ability to resolve specific differences in early developmental patterns of individual plants. A later modification of the Feekes scale (Zadoks et al. 1974) permitted quantification of the leaf number on the main stem and of the number of tillers present on the plant. For example, a plant with a score of 15.23 has five fully expanded leaves on the main stem and three tillers. This system tells the number of leaves on main stem and the number of tillers on the plant, but it did not indicate identity.

Haun (1973) devised another system where a wheat plant with a score of 6.2 had six leaves fully extended with the seventh leaf two-tenths of the length of the sixth. This system did not include a tillering index and occasionally, the upper leaf was not fully expanded but already longer than the preceding leaf. Another system for naming tillers on the main stem of grasses was used by Jewiss (1972). He named the tiller which developed in the axil of the first foliar leaf "T1", that from the second leaf "T2", and so on. Klepper et al. (1982) combined and extended the ideas used by Haun (1973) and Jewiss (1972) to permit a very specific and detailed designation of the development of cereal seedlings. Leaves were

numbered acropetally beginning with the first foliar leaf (L1). Tillers borne on the main stem bore the number of the leaf that subtends them; thus T1 was produced from the bud in the axil of L1. The coleoptile was designated as L0; the coleoptilar tiller T0. In fact, the coleoptilar node was the second node on the axis (Avery 1930; Esau 1977), but since the lower node, the scutellar node, does not produce tillers, it has not been given a number in this system.

Each main stem tiller can produce secondary tillers from buds in the leaf axils on the tiller. Tertiary tillers can be produced from secondary tiller, and so on. Tillers arising from the axil of the prophyll have a zero as the second digit. For example, T1 might have two subtillers, T10 and T11; the former would be produced from the bud in the axil of the prophyll of T1 and the latter from the axil of the first leaf of T1. The system becomes complicated in the designation of tertiary tillers or higher order tillers, but it nonetheless permits a unique designation for each leaf and axillary tiller on the plant (Figure 1).

**Role of coleoptilar tiller**

The coleoptilar node may produce a coleoptilar tiller or it may skip the tiller. Peterson et al. (1982) reported that the outgrowth of a tiller from the coleoptilar node was influenced by both the seed and environmental factors. The coleoptilar tiller had the potential to form a secondary crown if the primary crown was damaged, which could be important in areas where frost damage is a recurring problem (Webb and Stephen 1936).

Lewis and Garcia (1979) reported that coleoptilar tiller of tall fescue had a far greater and more permanent effect both on dry matter yield and tiller number when compared to the

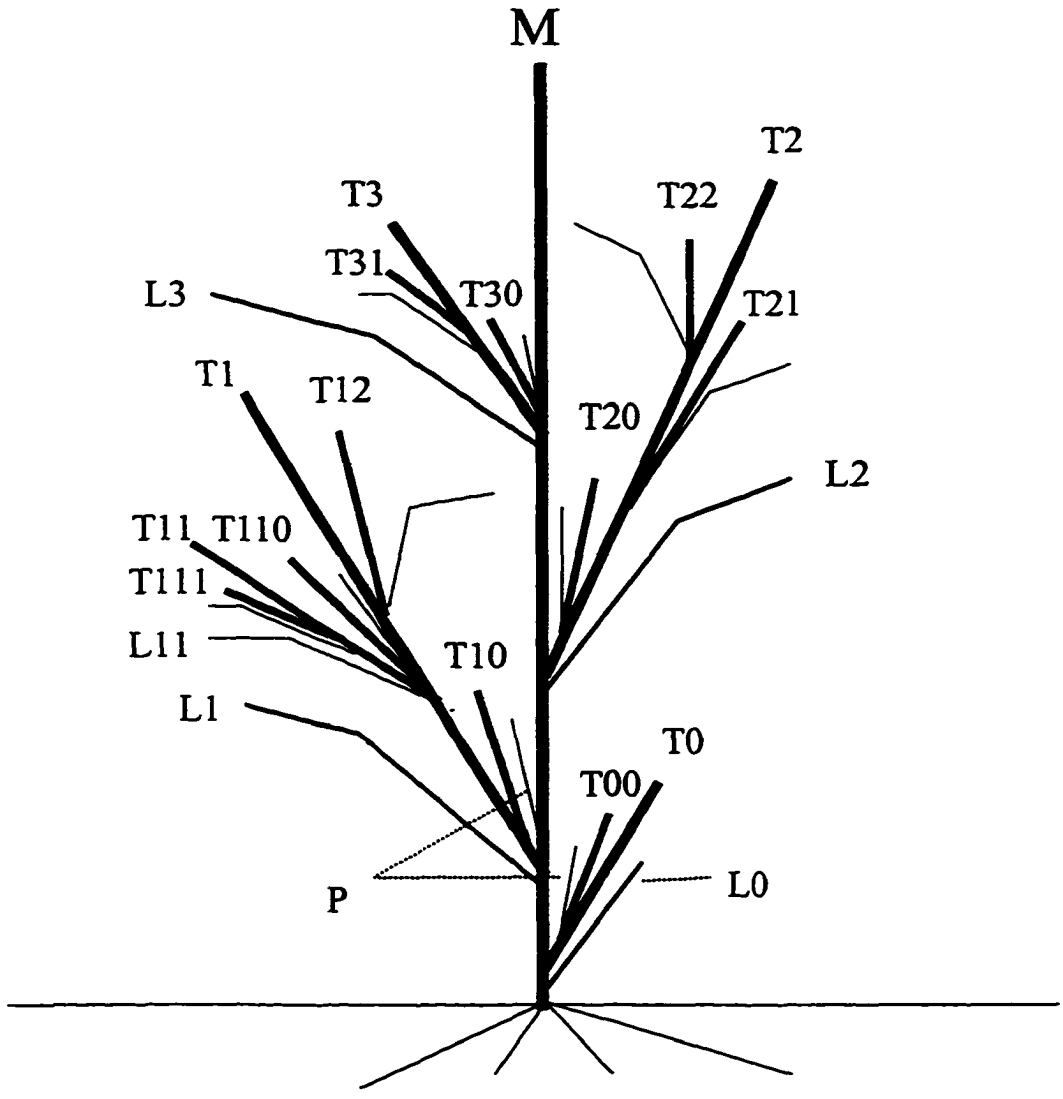


Figure 1. Woolly cupgrass seedling tillering pattern. M: main stem, L: leaf, T: tiller, L0: coleoptile, P: prophyll. Primary tillers carry one digit including T0, T1, T2, etc.; Secondary tillers carry two digits including T00, T10, T11, T20, T21, T22, T30, T31, etc.; Tertiary tillers carry three digits including T110, T111, etc.

plants without coleoptilar tillers. The initial difference of 28% in tiller numbers for the first harvest remained practically unchanged in the subsequent three harvests. Importantly, the percentage difference in dry matter yield between plants with and without a coleoptilar tiller remained more or less constant at about 22% from the second harvest onwards. On the contrary, the reports of the coleoptilar tiller contribution to grain yields were not consistent.

Rawson (1971) reported that wheat grain yields were directly related to age of the primary true-leaf tillers, but the primary and secondary prophyll tillers were much lower yielders than expected from age. He concluded that prophyll tillers, and particularly the coleoptilar tiller, do not yield as well as synchronous true-leaf tillers. Cannell (1969b) reported that 13% grain yield advantage of a new variety Deba Abed over an old variety Spratt Archer was mainly accounted for by the difference in production by the coleoptilar tiller.

## Factors affecting tiller development

Tiller development and the number of tillers produced by a grass plant is determined both by plant genetics and by environmental factors.

### Temperature and light intensity

The effect of environment on tillering in grasses has been extensively reviewed by Langer (1963). The higher the light intensity under which plants are grown, the higher the growth and tillering rates. By comparing plants at the same stage of leaf development on the main stem, Mitchell (1953a) showed that an increase in light intensity increased tiller number and the tillering rate which were brought about by increased photosynthesis. Low

temperature retarded the rate of leaf formation more than tillering so that apical dominance was less and the number of tillers present at a given stage of leaf development on the main stem was greater than at high temperatures.

Friend (1965) also reported that the rate of leaf emergence on individual axis and the rate of tillering increased as light intensity increased over the range 200 to 2500 ft-c. As the rate of tillering increased more than the rate of leaf emergence, apical dominance was reduced. An increase in temperature over the range 10 to 25 C also increased the total number of leaves and tillers, but the rate of leaf emergence was stimulated more than the rate of tillering so that apical dominance was increased.

**Carbohydrate supply and utilization**

The effects of the environment on the relationship between leaf and tiller production are attributed to the close dependence of tillering on the assimilate supply (Friend 1965). Cannell (1969a, 1969b) has shown that the primary tiller located at the coleoptilar node in barley is a shoot with particular sensitivity to environmental conditions. Coleoptilar tiller appearance is thought to be controlled by carbohydrate availability and utilization. Small seed size, excision of the first leaf, excision of the endosperm and low photosynthetic photon flux density (PPFD) all reduced coleoptilar tiller production in winter wheat (Peterson et al. 1982). When seedlings were grown at high temperatures, the bud in the coleoptilar axil failed to develop due to a high growth rate and subsequent use of endosperm reserves (Taylor and McCall 1936).

High N decreased coleoptilar tiller production by enhancing main stem growth (Frank and Bauer 1982). Leaf and root elongation rate affected carbohydrate availability for

coleoptilar tiller production. Skinner and Nelson (1994) reported that tall fescue with a high leaf elongation rate produced fewer tillers than a low leaf elongation rate population which produced 34% less root mass than the former. This was consistent with the hypothesis that rapid growth inhibits coleoptilar tiller production.

**Moisture**

Krenzer et al. (1991) reported that soil moisture had a significant impact on T0 formation, but not on T1, T2, or T3 in winter wheat. The low moisture plants did not form any T0, compared to 35.7% T0 formation for the high moisture plants. The phyllochron interval increased under a low moisture regime compared to a high moisture regime, and delayed tiller emergence in comparison to adequate moisture (Krenzer et al. 1991). Wheat growing in dry, crusted, or otherwise unfavorable seedbeds, usually had T2 as the first tiller produced (Klepper et al. 1982).

**Nutrients**

Elements plants require for growth are usually classified as macronutrients or micronutrients, according to the concentration in plant tissue. Nutrient availability affects the overall plant growth and tiller production. Nitrogen most dramatically influences vegetative growth in grass species. Longnecker et al. (1993) reported that low nitrogen levels reduced the wheat growth rate and delayed tiller emergence. The form of nitrogen also influenced tiller production and yield of some wheat varieties. A 1:1 ratio of nitrate and ammonium resulted in a larger root mass, more tillers and a larger yield (Wang and Below 1992). They concluded that a larger root mass resulted in better overall plant growth and increased tiller production.

**Plant density**

Tiller reduction, caused by high plant density, has been observed in wheat (Pugkridge 1968), barley (Kirby and Faris 1972), and ryegrass (Kays and Harper 1974). The effect may be attributed to competition for resources from both the above- and below-ground environments. Increased shading caused by higher densities not only reduces energy availability but also changes the red/far-red ratio of the light low in the canopy. A reduction in either the PPFD or the red/far-red ratio can decrease tillering (Casal et al. 1985; Deregibus et al. 1983; Mitchel 1953b).

**Herbicide tolerance and resistance**

Selectivity allows herbicides to control unwanted plants, weeds and volunteer crops, and not damage crops. Herbicide selectivity may result from differences in the plant, herbicide placement and time of application, and the addition of protectants or safeners. Herbicide tolerance is the inherent ability of a species to survive and reproduce after herbicide treatment (WSSA 1998). When tolerance comparisons were made either among weeds or among weeds and crops, differences were typically attributed to absorption, translocation or metabolism (Cottingham and Hatzios 1992; Green et al. 1992; Molin and Khan 1996; Risley and Oliver 1992). Herbicide tolerance sometimes involves more than one of the above mechanisms

Since triazine-resistant common groundsel (Senecio vulgaris L.) was first discovered in 1968 by Ryan (1970), herbicide resistant weeds have received considerable attention. Herbicide resistance is the inherited ability of a plant to survive and reproduce following

exposure to a dose of herbicide normally lethal to the wild type (WSSA 1998). Resistance may be naturally occurring or induced by such techniques as genetic engineering or selection of variants produced by tissue culture or mutagenesis. Altered herbicide target sites have been reported as responsible for herbicide resistance for many herbicide families in various weed species (Gimenez-Espinosa et al. 1996; Leach et al. 1995; Preston et al. 1996). Weeds developing cross-resistance to herbicides in the same family can be expected. This is mostly true for resistance attributed to an altered target site.

Weeds demonstrating multiple resistance usually have evolved multiple mechanisms of resistance. Preston et al. (1996) reported that a Lolium rigidum biotype showed resistance to herbicides from nine chemical classes with five modes of action. This biotype contained altered target sites for acetolactate synthase (ALS) and acetyl-coenzyme A carboxylase (ACCase) inhibiting herbicides. In addition, this biotype had an enhanced capacity for detoxification of the herbicides chlorotoluron (*N'* –(3-chloro-4-methylphenyl)-*N,N*-dimethylurea), simazine (6-chloro-*N,N'*-diethyl-1,3,5-triazine-2,4-diamine), chlorsulfuron (2-chloro-*N*-[[[4-methoxy-6-methyl-1,3,5-triazin-2-yl)amino]carbonyl]benzenesulfonamide), and diclofop-methyl (methyl 2-[4-(2,4-dichlorphenoxy)phenoxy]propanoate). Cytochrome P-450 monooxygenase inhibitor studies suggested that different cytochrome P-450 isozymes were responsible for the enhanced metabolism of different herbicides (Preston et al. 1996).

**Apical dominance and sublethal herbicide dose responses of plants**

Dose response studies showed that weed responses to low herbicide doses were variable when compared to high dose responses, and were dependent on the plant developmental

stage. Stunted main stems and prolific tillering seemed to be common responses of grasses to sublethal rates of many herbicides. (Caseley 1972; Chao et al. 1993; Derr et al. 1985; Pullins 1995).

Apical dominance may be defined as the control exerted by the shoot apex over the outgrowth of the lateral buds (Cline 1994). The lateral buds may be inhibited by apical dominance of the main apex shortly after their formation or after a brief period of growth, and remain as axillary buds unless the main apex is removed (Steeves and Sussex 1989). Although the elongation of the buds may be inhibited, they remain metabolically active (Stafstrom 1995). If the shoot apex is subsequently decapitated, apical dominance is released and one or more of the lower axillary buds begin to elongate. Robertson et al. (1989) reported that the scale leaves of quackgrass (Agropyron repens) rhizomes contribute to apical dominance by inhibiting the initial development of axillary buds. Removal of scale leaves promoted an initial burst of growth within the axillary buds.

Sublethal herbicide doses stopped the main shoot growth of grass and increased tillering (Caseley 1972; Chao et al. 1993; Derr et al. 1985). Chao et al. (1993) reported that prolific tillering in the imazamethabenz ((±)-2-[4, 5-dihydro-4-methyl-4-(1-methylethyl)-5-oxo-1 *H*-imidazol-2-yl]-4(and 5)-methylbenzoic acid (3:2))-treated wild oat (Avena fatua) plants resulted from the stimulated resumed growth of tillers and the increased initiation of tiller buds. Enhanced tillering mainly resulted from the release of apical dominance due to the inhibition of the main stem growth with the herbicide treatment. Chao et al. (1994) demonstrated that the radioactivity measured in the tillers of decapitated wild oat was 2.6-fold that in the tillers of intact plants 96 h after $^{14}$C-imazamethabenz treatment following

decapitation. This supported the hypothesis that main shoot apical dominance limits translocation of herbicide to tillers, rendering the tillers less susceptible to growth inhibition by the herbicide.

**Mechanical role of leaf sheath**

The stem (culm) of a grass consists of a series of nodes and internodes with a single leaf borne at each node (Bierhorst 1971). The leaf has two morphologically distinct regions, a free blade and a tubular sheath. The leaf growth occurs while the leaf is enclosed in the pseudostem (the rolls of older leaves). The leaf sheaths contribute significantly to the flexural rigidity of their enveloped internodes. (Niklas 1990). Mechanical analysis of the culms of Avena sativa indicates that as the culm matures and increases in overall height, internodes are mechanically supported by enveloping leaf sheath. Older, more basal internodes rigidify over time and their leaf sheaths play a lessening mechanical role over time (Niklas 1990). Niklas (1998) demonstrated that Arundinaria tecta leaf sheaths contributed 33% of the overall bending stiffness and 43% of the overall torsional stiffness of stem segments which help stem to resist bending and twisting forces.

Other than the role of support, the influence of leaf sheath on tiller bud development and growth have not been well documented. Williams et al. (1975) examined tiller bud initiation under the microscope and reported that the physical constraint of tiller buds by surrounding leaf sheaths, in combination with environmental factors, might determine the tillering pattern of wheat. Williams and Metcalf (1975) found that the application of additional constraint on wheat seedlings prevented the emergence of all tillers, yet, the removal of coleoptile allowed

the coleoptilar tiller to grow vigorously. Scale leaves inhibited the development of axillary buds of quackgrass rhizomes. Removal of scale leaves promoted an initial burst of bud growth regardless the existence of the apex. However, the aqueous extracts of scale leaves did not inhibit sprouting in denuded rhizomes (Robertson et al. 1989). The authors did not mention the physical constraint of scale leaf, yet, the possibility of scale leaf restricting tiller emergence due to the physical constraint should not be ignored.

**Tillage effects on weed seedbanks and populations**

Tillage affects weed species composition and depth of seed distribution in soil (Teasdale et al. 1991; Wrucke and Arnold 1985; Yenish et al. 1992). Changes in tillage can have a significant effect on weed populations and thus weed control (Ball and Miller 1990; Egley and Williams 1990). Tillage affects the soil seedbank by moving weed seeds buried deep in the soil nearer to the soil surface and seeds from the soil surface deeper in the soil thus increasing seed longevity and potentially prolonging the life of the seedbank. However, greater weed emergence in cultivated, compared to undisturbed plots, resulted in a faster decline of the soil seed reserves in the cultivated plots (Cardina et al. 1991; Egley and Williams 1990). Ogg and Dawson (1984) found that the seedbank response to tillage was species dependent; emergence of four broadleaf weed species was stimulated, three broadleaf species were unaffected, and emergence of one grass species was depressed by tillage. No-tillage systems typically had higher populations of small-seeded annual weeds while tillage systems using moldboard plows had more large-seeded annual weeds. Roberts and Potter (1980) demonstrated that weed emergence was dependent on the timing of cultivation

relative to rainfall.

The following research explores the mechanisms responsible for woolly cupgrass surviving nicosulfuron by initiating new tillers. New tiller emergence following herbicide applications has been observed in several grasses, but no decisive mechanisms were reported. Little information about woolly cupgrass germination responses to tillage and preemergence herbicides is available. This information will hopefully, provide a solid interpretation for tiller regrowth and result in better management of woolly cupgrass.

# TILLER DEVELOPMENT AND NICOSULFURON TRANSLOCATION IN WOOLLY CUPGRASS (Eriochloa villosa)

A paper to be submitted to Weed Science

Ming-Chung Liu and Micheal D. K. Owen

## ABSTRACT

Woolly cupgrass has a strong tillering ability. In 30 days it produced seven primary tillers, fourteen secondary tillers, and many tertiary tillers when grown in the greenhouse. Tiller bud initiation was much earlier than tiller emergence. Microscopic studies showed that an 11-day-old woolly cupgrass seedling with the first tiller emerged had already initiated nine tiller buds including five primary tiller buds and four secondary tiller buds. Secondary tiller buds were initiated from the primary tiller even before the primary tiller had emerged. Vascular connection between the main stem and the tillers was examined under a light microscope. No evidence was observed suggesting that there was a physical barrier preventing the translocation of herbicides from the main stem. Autoradiographic studies showed that $^{14}$C-nicosulfuron was translocated to every tiller bud regardless of herbicide placement on the plant. The mechanism(s) responsible for woolly cupgrass tiller emergence following nicosulfuron application was not likely due to the herbicide restricting the tiller buds. The strong tillering ability which caused the initiation of numerous tiller buds at the time of nicosulfuron application may contribute to tiller regrowth and new tiller initiation.

However, other mechanisms may be important and further research is required.

**Nomenclature:** Nicosulfuron, 2-[[[[(4,6-dimethoxy-2-pyrimidinyl)amino]carbonyl] amino]sulfonyl]-*N*,*N*-dimethyl-3-pyridicarboxamide; woolly cupgrass, Eriochloa villosa (Thunb.) Kunth. # ERBVI.

**Additional index words:** Herbicide translocation, paraffin sectioning, tiller initiation, vascular connection, ERBVI.

## INTRODUCTION

Woolly cupgrass tillers aggressively (Strand and Miller 1980). Owen (1990) reported that woolly cupgrass, under non-competitive environments, could produce 59 tillers per plant at maturity. Since tiller formation is an important component in the production of cereal grain as well as weed seeds, the tillering ability of woolly cupgrass was studied in detailed.

Haun (1973) developed a system to describe the developmental stage of grass seedlings. In this system, wheat (Triticum aestivum L.) with a Haun stage of 6.2 has six leaves fully expanded with the seventh leaf two-tenths of the length of the sixth leaf. This system did not include a tillering index. Jewiss (1972) named the tiller of grasses which developed in the axil of the first foliar leaf "T1", that from the second leaf "T2", and so on.

Klepper et al. (1982) combined and extended the ideas used by Haun (1973) and Jewiss (1972), to permit a very detailed designation of the cereal grain seedling development. Leaves are numbered acropetally beginning with the first foliar leaf (L1). Tillers borne on the main stem bear the number of the leaf that subtends them; thus T1 is produced from the bud in the axil of L1. The coleoptile is designated as L0, and the coleoptilar tiller T0.

Skinner and Nelson (1994) compared tiller production between tall fescue (Festuca arundinacea) populations with low and high leaf elongation rates (LER). They found the appearance of the coleoptilar tiller was extremely important in determining total tiller production since its progeny comprised more than one-half the potential tillers on a plant. Coleoptilar tiller appearance was affected by carbohydrate availability and utilization. Small seed size, excision of the first leaf, excision of the endosperm and low PPFD all reduced coleoptilar tiller production in winter wheat (Peterson et al. 1982).

McCall (1934) suggested that low respiration and slow growth allowed more stored nutrients from the endosperm to be available for the development and growth of vascular connections to the coleoptilar bud. When seedlings were grown at high temperatures, the use of endosperm reserves and growth were rapid, thus causing the failure of the buds in the coleoptilar axil (Taylor and McCall 1936). High N also decreased coleoptilar tiller production, presumably by enhancing main stem growth (Frank and Bauer 1982).

Skinner and Nelson (1994) expected that more rapid leaf elongation in the high LER population would require more carbohydrates and thus reduce coleoptilar tiller production relative to the low LER population. They found the low LER population produced twice as many coleoptilar tillers as the high LER population after four tiller production cycles. However, factors that altered carbohydrate supply or utilization, including seed size, photosynthetic rate, and respiration rate, were not different between high LER and low LER populations. They suggested that some other factor regulated coleoptilar tiller appearance.

Woolly cupgrass survived applications of nicosulfuron by initiating new tillers (Pullins 1995). The main stem was killed by the nicosulfuron but tillers emerged and developed into

healthy plants. The tillering ability of woolly cupgrass was suspected to be one of the mechanisms which might contribute to tiller regrowth or new tiller initiation, yet had not been examined. Tiller emergence has long been the accepted criterion for the presence of tillers in agronomic studies, and even in physiological studies of apical dominance in the Poaceae. However, tiller buds have been growing for days or even weeks before they do emerge (Williams et al. 1975). The recommended woolly cupgrass growth stages for nicosulfuron application was 5 to 10 cm tall with less than three tillers (CPR 1996). However, the number of tiller buds at these growth stages has not been reported in woolly cupgrass. Data on tiller bud initiation is needed to determine whether those unemerged tiller buds contributed to tiller regrowth, and therefore woolly cupgrass survival of nicosulfuron application.

The new tillers likely did not receive herbicide directly from the application but herbicide could translocate from the main stem (Chao et al. 1994). The tiller buds might be dormant at the time of herbicide application due to the apical dominance imposed by the main stem and thus were unaffected. However, Chao et al. (1994) reported that when apical dominance was released, more imazamethabenz ((±)-2-[4, 5-dihydro-4-methyl-4-(1-methylethyl)-5-oxo-1 *H*-imidazol-2-yl]-4(and 5)-methylbenzoic acid (3:2)) was translocated to the tillers of wild oat (Avena fatua), thus increasing the phytotoxicity. Tiller regrowth might be due to the interruption of translocation, which reduced the herbicide concentration in the tiller buds. This has not been confirmed.

The objectives of these experiments were to investigate the mechanisms responsible for tiller regrowth or tiller initiation in woolly cupgrass following nicosulfuron application. The tillering ability of woolly cupgrass and the vascular connection between the main stem and tillers were also examined.

## MATERIALS AND METHODS

Woolly cupgrass seeds used for the following experiments were collected from native woolly cupgrass populations in a corn field at Ogden, Iowa, in September 1995. Seeds were air-dried and stored at 4 C until used.

### Tillering pattern

The experiment was conducted in the greenhouse and repeated. The first experiment received natural illumination and temperature fluctuated between 20 and 32 C. Radiation in the second experiment was supplemented with 1000 watt high pressure sodium lamps and maintained at 450 to 550 $\mu$mol $m^{-2}$ $s^{-1}$ of PPFD at the canopy level with a 16-h photoperiod. Radiation could reach 850 $\mu$mol $m^{-2}$ $s^{-1}$ of PPFD at midday. Day and night temperatures were 28 C and 20 C, respectively. Greenhouse conditions for the second experiment were used for the rest greenhouse experiments in our study. Woolly cupgrass was grown in 15-cm-diam. ceramic pots with potting soil mixture of soil:perlite:peat (2:1:2 by wt.). Each pot was sown with 3 seeds and thinned to one plant per pot 7 days after planting (DAP). Forty plants and twenty plants were used to calculate the percentage of tiller emergence in the first and the second experiments, respectively. Plants were fertilized weekly with 100 ml per pot of 20 ppm 20:20:20 N:P:K.

Leaves were marked to identify tillers when leaf blade was fully expanded. Tillers were identified according to the system developed by Klepper et al. (1982). Experiments were terminated 30 DAP. The percentage of individual tiller was calculated. In the second experiment, the emergence dates of leaves on the main stem and the emergence dates of primary and secondary tillers were also recorded.

**Microscopic study of tiller development**

Woolly cupgrass plants were grown in greenhouse conditions as described previously. Woolly cupgrass seeds were planted in 10-cm-diam. pots and four pots were sown with excess seeds every day for 9 consecutive days and thinned to 5 plants per pot 7 DAP. Plants were evaluated 13 days after the first planting day. Thus, there were 9 different seedling stages, each from 5 to 13 days old. Twenty plants from each seedling stage were harvested and fixed in FAA (37% formaldehyde, 50 ml, glacial acetic acid, 50 ml, and 50% alcohol, 900 ml to make 1000 ml of the mixture) for paraffin sectioning preparations and subsequent microscopic study.

The technique of paraffin sectioning was modified from Berlyn and Miksche (1976) and Horner (1994). The stems of woolly cupgrass seedlings were excised with the FAA at the first leaf collar and top of the mesocotyl. The stem segments were immediately fixed with FAA in 20-ml vials for 24 h. Stem segments were dehydrated in a series of ethanol and tertiary butyl alcohol mixtures, then passed through at least six changes of paraffin[1] and embedded. Ten-*u*m-thick sections were cut transverse to the axes on a microtome[2] for light microscopy evaluation. The sections were mounted on glass slides, de-waxed in xylene, hydrated in a graded ethanol series to 50% ethanol, and double stained with 1% safranin in

50% ethanol for 4 h and with 1% fast green in 95% ethanol for 10 s. Slides were then dehydrated in a graded ethanol series to xylene and Permount[3] was added. Samples were observed with a Leitz Orthoplan microscope[4] and images were recorded on Kodak Technical Pan film[5].

**Herbicide translocation between the main stem and the tillers**

Woolly cupgrass plants were grown in the growth chamber in a potting soil mixture of soil:perlite:peat (2:1:2 by wt.). Radiation was maintained at 250 *u*mol $m^{-2}$ $s^{-1}$ of PPFD at the canopy level with a 16-h photoperiod. Day and night temperatures were 30 and 20 C, respectively, and day and night relative humidities were maintained at 65 and 85%. Woolly cupgrass seeds were planted in 10-cm-diam. pots and thinned to one plant per pot 7 DAP. Ten to 14 day-old seedlings with 1 to 3 tillers were treated with $^{14}$C-labeled nicosulfuron. Plants were sprayed with 35 g ai $ha^{-1}$ nicosulfuron and X-77[6] (0.25% by vol.) and allowed to dry for 30 min. Five 2-*u*l droplets of $^{14}$C-nicosulfuron were applied to the most recent fully-expanded leaf on the main stem. Each 10-*u*l application contained approximately 100,000 disintegration per minute (DPM) of 2-pyrimidine-$^{14}$C-labeled nicosulfuron. Treated plants were harvested at 4 and 24 h after treatment (HAT). Tillers were separated from the main stem, and stored at -10 C for 24 h. Tillers were oxidized with a sample oxidizer[7] and the resultant $^{14}CO_2$ captured in 15 ml of carbon absorbent/scintillation cocktail[8]. All scintillation vials were measured for radioactivity with a liquid scintillation spectrophotometer[9]. Experiments were a completely randomized design (CRD) containing five plant stages with four replications and were conducted twice.

**Autoradiographic study**

Woolly cupgrass plants were grown in the greenhouse as described previously. Twelve-day-old plants were treated with $^{14}$C-nicosulfuron as described previously except that the $^{14}$C-nicosulfuron was applied to the third leaf on the main stem or the first true leaf on tiller 1, respectively, in order to examine nicosulfuron translocation. Plants were fixed with FAA 24 h after treatment for autoradiographic study. Stems were fixed, dehydrated, and embedded with paraffin as previously described. Serial cross sections of the stems were cut on a microtome, mounted on glass slides, and coated with Kodak NTB-2 liquid emulsion[10] under a red safelight. The slides were dried, then packed in light-tight boxes containing a desiccant, and stored in a 4 C refrigerator for 7 d. At the end of the exposure period, slides were developed in Kodak Dektol (1:1 with $H_2O$) developer[11] for 4 min, washed 30 s in $H_2O$, fixed in Kodak fixer[12] for 4 min, and washed in running tap water for 10 min. Slides were then dehydrated in a graded ethanol series to xylene, and Permount was added, followed by a cover slip. Slides were observed and photographed with a Leitz Orthoplan microscope. Images were recorded on Kodak Technical Pan film.

## RESULTS AND DISCUSSION

**Tiller pattern**

Woolly cupgrass seedlings took 3 to 4 days to emerge when grown in the greenhouse. The coleoptilar node was pushed near the soil surface by elongation of the mesocotyl. This emergence pattern is different from that reported on wheat by Webb and Stephens (1936), who stated that the coleoptilar node would remain in the seed and stay at the depth where the

seed was planted. The coleoptilar tiller of woolly cupgrass was not observed. The first tiller emergence, which could be either T1 or T2, usually occurred 10 to 12 DAP. When T2 emerged ahead of T1, the development of T1 was affected by the rapid growth of the main stem, which presumably used up most of the available carbohydrate supply. Factors that reduce carbohydrate supply or increase carbohydrate consumption reduce coleoptilar tiller production (Frank and Bauer 1982; Peterson et al. 1982; Taylor and McCall 1936). Skinner and Nelson (1994) reported that tall fescue with a high LER produced fewer tillers. The rapid growth of woolly cupgrass seedlings not only inhibited the coleoptilar tiller production but also affected T1 development.

At 30 DAP, the Haun stage of woolly cupgrass in the first and the second experiments was 10.2 and 10.6, respectively. Woolly cupgrass produced primary tillers up to T7 (Table 1). The percentage of plants that produced T1 through T6 tillers was 100%, and the second experiment had a higher percentage of T7 production. Secondary tillers represented more than 50% of the total tillers. T10 initiated from the axil of prophyll on T1 and the mitiation and growth of T10 may be affected by limiting availability of carbohydrates when the seedlings were small.

All plants produced T20, T30 and T40. Prophyll tillers initiated and grew normally when seedlings were healthy and energetic except that T10 emergence was delayed or absent. True leaf tillers never failed to initiate and emerge. Plants produced more tertiary tillers in the second experiment than in the first experiment, a difference likely caused by the higher PPFD due to supplemental artificial light for the second experiment.

Primary tillers emerged at a steady rate of 2.8 days per tiller after the first tiller emerged (Figure 1). Secondary tillers at different primary tillers also increased at similar rate except that the appearance of T10 was delayed (Figure 2). Klepper et al. (1982) reported that the production of leaves and tillers on primary and higher order tillers of vegetative grass plants appeared to be synchronized with leaf development on the main stem. This made the pattern of tiller appearance predictable. In our studies, the rate of woolly cupgrass tiller production was high, but the number of tillers was far less than the estimates in perennial ryegrass by Neuteboom and Lantinga (1989) who assumed the potential number of tiller could double for each main stem phyllochron. Tiller production was not highly synchronized with leaf development on the main stem in woolly cupgrass, presumably due to limiting growth factors.

**Microscopic study of tiller development**

This study was conducted to examine the tiller development of woolly cupgrass seedlings at the stages when the woolly cupgrass plants are most susceptible to nicosulfuron. Seedling characteristics of woolly cupgrass were measured when they were fixed for microscopic study (Table 2). Tiller 1 emerged 11 DAP and tiller 2 emerged one day later; this was consistent in all our studies. Tiller bud initiation was much earlier than tiller emergence. By examining the serial sections from different plant stages under the light microscope, leaves, tiller buds, and root primordium could be identified and recorded (Figure 3). At 5 DAP, seedlings already had tiller 1 and tiller 2 initiated (Table 3). Fahn (1982) reported that a mature wheat embryo usually had three immature leaves, a shoot apex, and a coleoptilar bud enclosed by the coleoptile. Although woolly cupgrass embryos were not

examined in our study, no coleoptilar bud was found regardless of plant stage suggesting that the coleoptilar bud did not pre-exist in the embryo.

Tiller buds developed rapidly. At 8 DAP, five tiller buds developed, including four primary tillers and one secondary tiller (T10). At 11 DAP, when T1 emerged, eight to nine tiller buds had initiated. The primary tiller buds initiated as many as T5; for the secondary tiller buds, two tillers (T10 and T11) had initiated on T1 and two tillers (T20 and T21) had initiated on T2. At 13 DAP, there were nine to thirteen tiller buds initiated, while only two tillers had actually emerged.

From the examination of different aged plants, primary tillers initiated first and secondary tillers initiated from the axils of leaves on the primary tillers. The secondary tiller buds initiated even before the primary tillers emerged. Twenty to 45% of woolly cupgrass plants in the previous studies did not produce T10 (Table 1); however, through microscopic examination, the bud for T10 always existed 8 DAP. The growth of the T10 bud was delayed or aborted because of environmental factors. Rapid growth of the main stem presumably consumed most of available assimilate and may partially explain the delay or abortion of T10 in this study.

Vascular connections between the main stem and tiller buds were examined to see whether there was a physical barrier which could prevent herbicide translocation. The vascular connection between tiller 3 and the main stem on a 13-day-old plant was visible (Figure 4). The vascular bundles appeared at one side of the stele and gradually moved further to the edge of the stele forming clusters, which were identified as the tiller stele. Tillers separated gradually from the main stem and became independent. Lersten (1987)

described that the tiller buds of wheat made early vascular connections with the adjacent nodal plexus and bundles descending from higher leaves. Tillers were therefore connected to the entire vascular system of the main axis. By examining sections from different aged woolly cupgrass plants, no interruption or physical barrier to vascular connections was observed. Thus tillers are not physiologically independent of the main stem at these growth stages and herbicides can translocate between the tillers and the main stem.

**Herbicide translocation and autoradiographic study**

Experiments were conducted to describe nicosulfuron movement between the main stem and tillers. The radioactivity detected in T1, T2, and, in some plants, T3 proved that $^{14}C$ translocated from the main stem to tillers 4 h and 24 h after treatment regardless of plant age (Table 4). More $^{14}C$ translocated to tillers after 24 h than 4 h. Woolly cupgrass had already initiated many tiller buds at these growth stages. Autoradiographs showed when $^{14}C$-labeled nicosulfuron was applied to the main stem, $^{14}C$ was detected in every tiller bud 24 h after treatment. When $^{14}C$-labeled nicosulfuron was applied to the first tiller, $^{14}C$ was detected in the main stem and other tiller buds. Since the half-life of nicosulfuron in woolly cupgrass was greater than 72 h (Hinz and Owen 1996), and the metabolite of nicosulfuron would be trapped in the cell and not be expected to be in the vascular tissue (Strachen 1994, personal communication), the $^{14}C$ observed in the tiller buds was assumed to be the parent herbicide rather than a nicosulfuron metabolite.

Many herbicide translocation studies demonstrated that radio-labeled herbicide appeared below and above the treated leaf and represented herbicide that was translocated out of the treated leaf (Hinz and Owen 1996; Lym 1992; Nimbal 1995; Walls et al. 1993). Some

studies reported more radio-labeled herbicide in the leaf below the treated leaf than in the leaf above the treated leaf (Hinz and Owen 1996). Chao et al. (1997) further demonstrated $^{14}C$-imazamethabenz applied to the first wild oat leaf on T1, 50% $^{14}C$ translocated to T1, 25% to the main stem, 20% to roots, and 5% to all other tillers. This implied that herbicides could move from a sink leaf to a source leaf. The concept of assimilate sink and source could not ignore the fact that the whole vascular system was connected, and the herbicide could translocate to every part of the seedling.

In conclusion, the tiller growth following nicosulfuron application was not likely due to the interruption of herbicide translocation, since translocation studies showed that nicosulfuron reached tillers in 4 h and nicosulfuron was found in all unemerged tiller buds 24 HAT. The strong tillering ability of woolly cupgrass likely contributed to tiller re-growth or new tiller initiation. However, other factors should be examined to fully explain these observations.

## SOURCES OF MATERIALS

[1] Paraffin, Oxford Labware, St. Louis, MO 63103.

[2] Model AO 820, American Optical Company, Buffalo, NY.

[3] Permount, Fisher Scientific, Pittsburgh, PA.

[4] Leitz Orthoplan microscope, Leica Canada, Midland, ON.

[5] Kodak Technical Pan, Eastman Kodak Co., Rochester, NY 14650.

[6] X-77, Valent USA Corp., Walnut Creek, CA 95496.

[7] Model OX500, R. J. Harvey Instrument Corp., 123 Patterson St., Hillsdale, NT 07642.

[8] Carbon-14 Cocktail, R. J. Harvey Instrument Corp., 123 Patterson St., Hillsdale, NT 07642.

[9] Model 3801, Beckman, Fullerton, CA 92634.

[10] NTB-2, Eastman Kodak Company, P.O. Box 9558, New Haven, CT 06535.

[11] Kodak Dektol developer, Eastman Kodak Company, Rochester, NY 14650.

[12] Kodak fixer, Eastman Kodak Company, Rochester, NY 14650.

## ACKNOWLEDMENTS

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Table 1. Woolly cupgrass tiller production. Tiller numbers are expressed as a percentage of the presence on 40 plants in experiment 1 and on 20 plants in experiment 2.

| | Experiment 1 | Experiment 2 |
|---|---|---|
| | % | |
| Primary tillers | | |
| T0 | 0 | 0 |
| T1 | 100 | 100 |
| T2 | 100 | 100 |
| T3 | 100 | 100 |
| T4 | 100 | 100 |
| T5 | 100 | 100 |
| T6 | 100 | 100 |
| T7 | 62.5 | 95 |
| Secondary tillers | | |
| T10 | 55 | 80 |
| T11 | 100 | 100 |
| T12 | 100 | 100 |
| T13 | 100 | 100 |
| T14 | 70 | 85 |
| T20 | 100 | 100 |
| T21 | 100 | 100 |
| T22 | 100 | 100 |
| T23 | 92.5 | 100 |
| T24 | 20 | 10 |
| T30 | 100 | 100 |
| T31 | 100 | 100 |
| T32 | 75 | 95 |
| T33 | 2.5 | 0 |
| T40 | 97.5 | 100 |
| T41 | 57.5 | 75 |
| T50 | 15 | 55 |

Table 1. Woolly cupgrass tiller production. Tiller numbers are expressed as a percentage of the presence on 40 plants in experiment 1 and on 20 plants in experiment 2. (cont.)

| | Experiment 1 | Experiment 2 |
|---|---|---|
| | % | |
| Tertiary tillers | | |
| T100 | 2.5 | 5 |
| T110 | 47.5 | 80 |
| T111 | | 45 |
| T120 | 12.5 | 20 |
| T200 | 42.5 | 55 |
| T210 | 5 | 35 |
| T300 | | 25 |

Table 2. Haun[a] stage, plant height, and tillers of woolly cupgrass while fixed with FAA[b].

| Plant age[c] (DAP) | Haun stage[d] | Plant height[d] (cm) | Tillers emerged |
|---|---|---|---|
| 5 | 1.8±0.3 | 1.2±0.2 | |
| 6 | 2.2±0.2 | 1.8±0.2 | |
| 7 | 2.6±0.3 | 2.0±0.1 | |
| 8 | 3.1±0.3 | 3.3±0.2 | |
| 9 | 3.2±0.4 | 3.5±0.1 | |
| 10 | 3.2±0.3 | 4.0+0.3 | |
| 11 | 3.8±0.3 | 4.8±0.3 | T1 |
| 12 | 4.2±0.4 | 6.5±0.4 | T1, T2 |
| 13 | 4.2±0.4 | 6.8±0.3 | T1, T2 |

[a] Haun stage: leaf number on the main stem (Haun 1973).
[b] FAA: 37% Formaldehyde, Glacier acetic acid and 50% Alcohol (1:1:18 by vol.).
[c] DAP: Days after planting.
[d] Values are means ± standard deviations of the means.

Table 3. Tillers observed in different aged woolly cupgrass seedlings examined under light microscope[a].

| Plant age[b] (DAP) | No. of leaf primordium | Primary tiller bud[c] | | | | |
|---|---|---|---|---|---|---|
| | | T1 | T2 | T3 | T4 | T5 |
| 5 | 5 | X | X | | | |
| 6 | 6 | X | X | X | | |
| 7 | 6 | X | X | X | | |
| 8 | 7 | X | X | X | X | |
| 9 | 8 | X | X | X | X | |
| 10 | 8 | X | X | X | X | |
| 11 | 8 | X | X | X | X | X |
| 12 | 9 | X | X | X | X | X |
| 13 | 9 | X | X | X | X | X |

[a] Cross sections of plant tissues were prepared through paraffin sectioning.
[b] DAP: days after planting.
[c] "X" represents the presence of tiller bud. Five seedlings from each stage were examined. For higher tiller, the tiller bud initiation was confirmed when they were found at least on three seedlings.

Table 3. Tillers observed in different aged woolly cupgrass seedlings examined under light microscope[a]. (cont.)

| Plant age[b] (DAP) | No. of leaf primordium | Secondary tiller bud[c] | | | | |
|---|---|---|---|---|---|---|
| | | T10 | T11 | T20 | T21 | T30 |
| 5 | 5 | | | | | |
| 6 | 6 | | | | | |
| 7 | 6 | | | | | |
| 8 | 7 | X | | | | |
| 9 | 8 | X | | X | | |
| 10 | 8 | X | | X | | |
| 11 | 8 | X | X | X | X | |
| 12 | 9 | X | X | X | X | X |
| 13 | 9 | X | X | X | X | X |

[a] Cross sections of plant tissues were prepared through paraffin sectioning.
[b] DAP: days after planting.
[c] "X" represents the presence of tiller bud. Five seedlings from each stage were examined. For higher tiller, the tiller bud initiation was confirmed when they were found at least on three seedlings.

Table 4. Effect of time and plant age on distribution of $^{14}C$-labeled nicosulfuron in the woolly cupgrass tillers.

| Plant age (DAP)[a] | | 14 | 13 | 12 | 11 | 10 |
|---|---|---|---|---|---|---|
| Sampling time | Tillers | | | DPM[b] | | |
| 4 HAT[c] | T1 | 9.6± 2.7 | 8.3± 3.3 | 10.4±0.5(3)[d] | | |
| | T2 | 34.8± 9.1 | 91.8±53.4 | 35.5±10.1 | 29.6±20.3 | 16.3±6.3 |
| | T3 | 49.5±11.4 | 69.3±64.5 | 20.9(1) | | |
| 24 HAT | T1 | 30.5±10.9(3) | 39.6±19.7(3) | | | |
| | T2 | 69.7±15.1 | 134.9±46.4 | 65.0±6.5 | 67.8±22.6 | 37.2±14.4 |
| | T3 | 73.9±27.7 | 73.7±26.7 | 33.0(1) | | |

[a] DAP: Days After Planting.
[b] DPM: Disintegration Per Minute measured with a liquid scintillation spectrophotometer.
[c] HAT: hours after $^{14}C$-labeled nicosulfuron treatment.
[d] Mean ± Standard Deviation. Four woolly cupgrass seedlings were used for each treatment except if the replication number was indicated in the parenthesis.

## LIST OF CAPTIONS

Figure 1. Duration from sowing to primary tiller appearance on woolly cupgrass main stem and number of days (phyllochron) between the appearance of successive tillers. Bar represents standard deviation. Data are averaged over 20 plants except indicated in the parenthesis.

Figure 2. Duration from sowing to secondary tiller appearance on various woolly cupgrass primary tillers. T1 is the tiller initiated from the axil of L1 on the main stem. T1 with numbers on the X axis represent secondary tiller on T1, i.e., T10 is the tiller initiated from the axil of prophyll on T1. Bar represents standard deviation. Data are averaged over 20 plants except indicated in the parenthesis.

Figure 3. Woolly cupgrass stem cross sections from different-aged plants. A: cross section of a 5-day-old plant. 480 *u*m below the apex. Bar=200 *u*m. B: cross section of a 8-day-old plant. 1920 *u*m below the apex. Bar=400 *u*m. M: main stem; L0: coleoptile; L1: first leaf; T1: primary tiller; P: prophyll; T10: secondary tiller.

Figure 4. Woolly cupgrass vascular connection between the main stem and tiller 3. A-F are cross sections located on different positions of a 13-day-old seedling. A and B : Leaf 3 separated from the main stem; C and D : vascular bundles connected to tiller 3; E and F : Tiller 3 separated from the main stem. Bar=500 *u*m. L3: leaf 3; T1: tiller 1; T3: tiller 3; RP: root primordium.

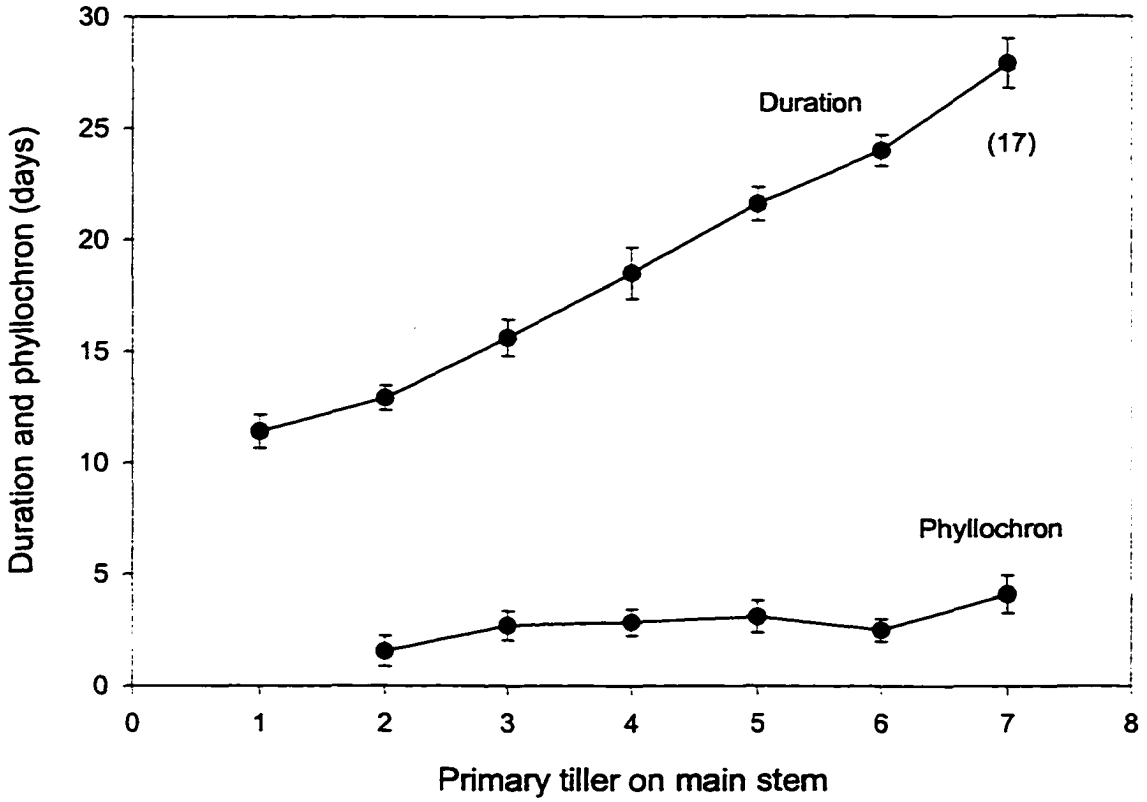


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Figure 1

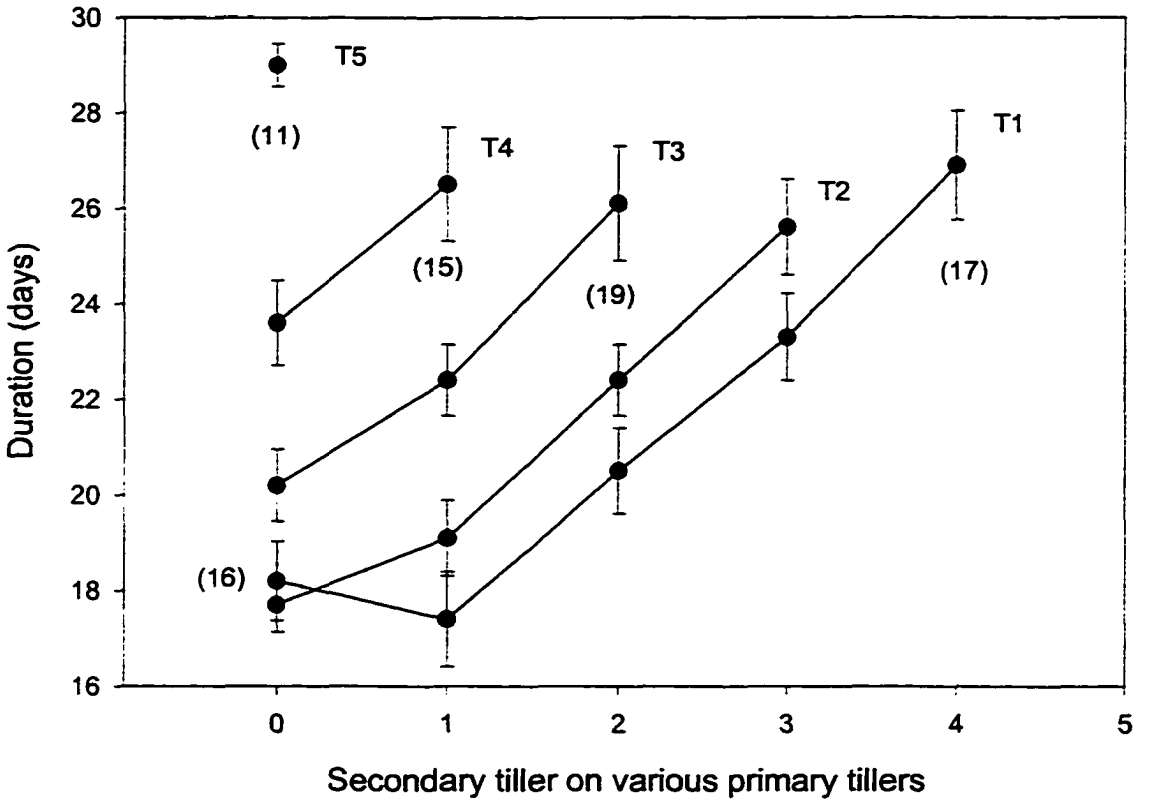


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Figure 2

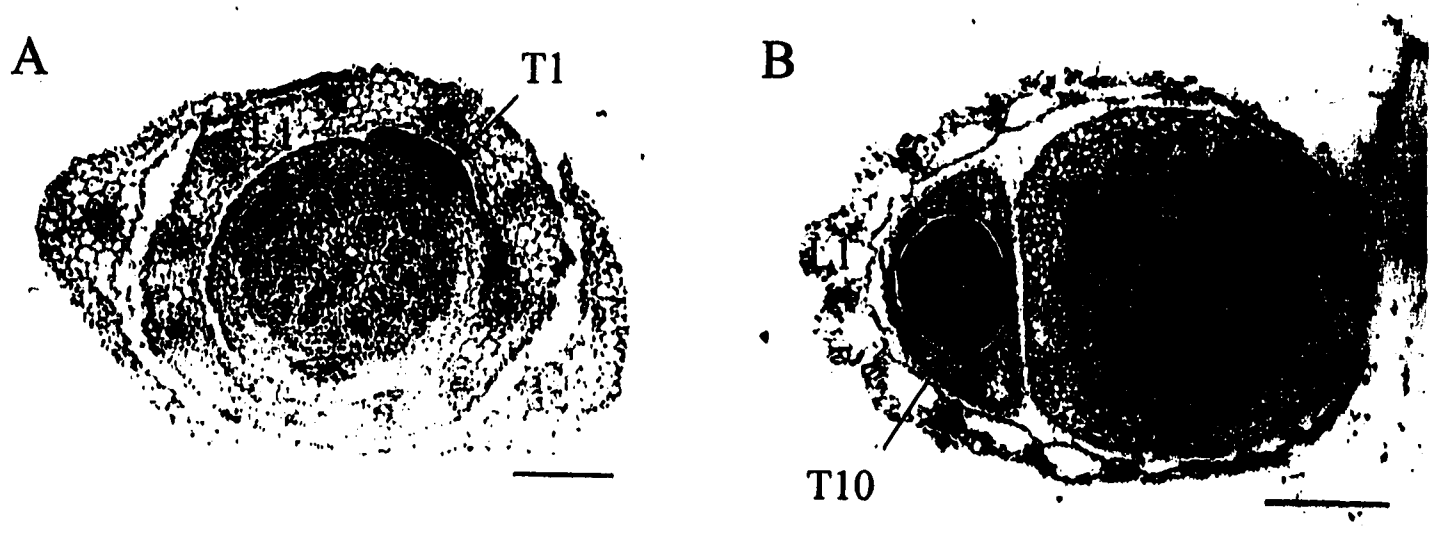


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Figure 3

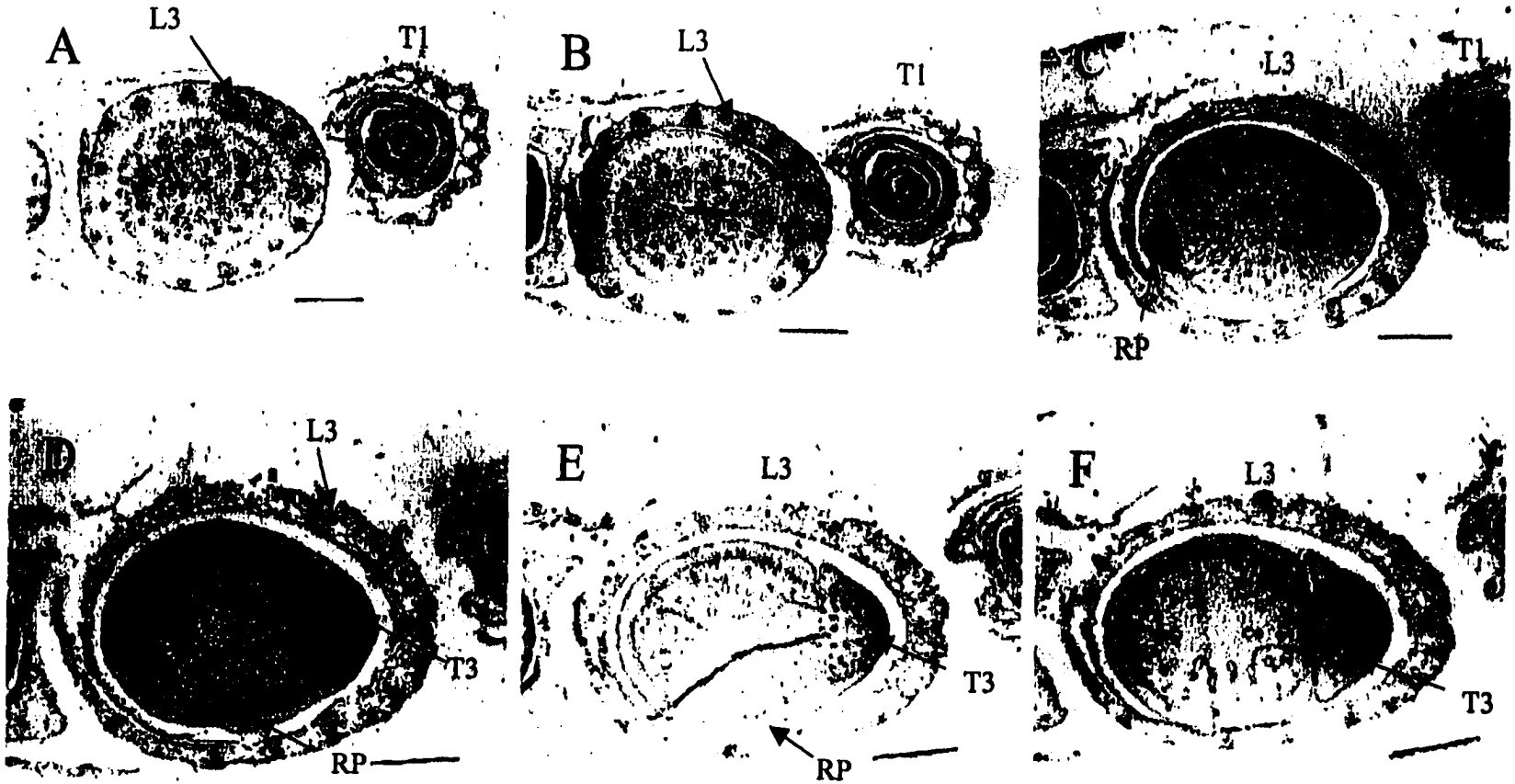


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Figure 4

# EFFECT OF SEED RESERVE UTILIZATION ON WOOLLY CUPGRASS (Eriochloa villosa) DEVELOPMENT

A paper to be submitted to Weed Science

Ming-Chung Liu and Micheal D. K. Owen

## ABSTRACT

Woolly cupgrass seeds are larger than many other weed seeds and may contribute to early tiller growth. Experiments examined woolly cupgrass seed reserve utilization and the effect of germination depth on seed reserve utilization and tiller production. Woolly cupgrass seeds were germinated on blotter papers in the growth chamber. The first experiment included three treatments: germination in the light with Hoagland's solution[1], in the light with water, and in the dark with water. Endosperm utilization and shoot and root growth over time were recorded. The second experiment included five planting depths of 1, 3, 5, 7, and 9 cm, and was conducted in the greenhouse. Endosperms lost weight rapidly until d 6 when weight loss slowed. Endosperm weight loss ceased after d 8 or d 10. Endosperms were considered depleted 8 to 10 d after germination, depending on the treatment. Endosperm weight loss was faster for light germination with Hoagland's solution than in the other two treatments. Woolly cupgrass seedlings grew faster and endosperm was depleted faster with light and nutrients. Deep planting significantly decreased the emergence rate and increased the time to emergence. Difference in residual endosperm may have affected the early

development of woolly cupgrass seedlings. Germination from 9 cm depleted the endosperm by the time of emergence. Deep planting significantly decreased shoot dry weight and tiller number 15 d after planting. Since the first tiller of woolly cupgrass usually appears at 10 to 12 d after planting, and the endosperm had been depleted before that, there was no endosperm to support the new tillers.

**Nomenclature:** Nicosulfuron, 2-[[[[(4,6-dimethoxy-2-pyrimidinyl)amino]carbonyl] amino]sulfonyl]-*N*,*N*-dimethyl-3-pyridicarboxamide; woolly cupgrass, Eriochloa villosa (Thunb.) Kunth. # ERBVI.

**Additional index words:** Endosperm, germination depth, seed reserve utilization, ERBVI.

## INTRODUCTION

Endosperm or seed reserve is the energy source for germination and early growth of grass seedlings (Dure 1960; Ingle et al. 1964). The influence of seed reserves on early seedling development and establishment has received considerable attention in cereal crops. Seed size affected seedling emergence (Lafond and Baker 1986), development (Bremner et al. 1963; Evans and Bhatt 1977), final plant size (Kincl 1975), and yield (Hampton 1981; Pinthus and Osher 1966). Seedling vigor was affected more by endosperm size than by embryo size in wheat (Triticum aestivum L.) seedlings (Bremner et al. 1963). A reduction in endosperm reserves, either due to seed damage or endosperm excision, retarded wheat seedling growth and development, including delaying the emergence of the first two leaves, tillers and inhibiting the outgrowth of the coleoptilar tiller (Chin and Aitkens 1976; Peterson et al. 1989). Plant dry weight, leaf numbers, and total number of tillers were all negatively

affected by endosperm excision (Peterson et al. 1989).

Seed reserves are important not only from the standpoint of supplying energy for early seedling growth, but also for supplying adequate leaf area for a more rapid growth rate (Cooper and MacDonald 1970). Germination depth was found to be the most important factor affecting seedling emergence of the individual crops. Deep planting decreased germination rates of many grasses and legumes, increased time to emergence, and decreased seedling vigor (Huang and Taylor 1993; Murphy and Arny 1939). These responses could be attributed to the consumption of more seed reserves by seedlings during emergence.

The early growth of a grass seedling can be divided into three stages according to energy source: heterotrophic, transitional, and autotrophic stages (Whalley et al. 1966). Murray and Cooper (1967) reported that the orchardgrass (Dactylis glomerata L.) green shoots which were capable of conducting photosynthesis developed long before the endosperm was completely utilized. Woolly cupgrass was found to survive applications of nicosulfuron, an acetolactate synthase (ALS, EC 4.1.3.18, also known as acetohydroxyacid synthase) inhibitor herbicide, by initiating new tillers in the field (Pullins 1995).

Woolly cupgrass has relatively large seeds compared to many other weed seeds, and the seed reserve was able to support woolly cupgrass seedling emergence from 9 cm (Owen 1990). New tiller emergence could be supported by the energy that remained in the endosperm if woolly cupgrass seeds germinated from shallow depths. The objectives of this study were to determine if woolly cupgrass seed reserves, under different conditions, could support the growth and emergence of new tillers and how germination depth affected seed reserve utilization and tiller production.

# MATERIALS AND METHODS

Woolly cupgrass seeds used for the following experiments were collected from native woolly cupgrass populations in a corn field at Ogden, Iowa, in September 1995. Seeds were air-dried and stored at 4 C until used.

**Effect of light and nutrients on endosperm utilization by woolly cupgrass**

Twenty woolly cupgrass seeds were germinated in plexiglass boxes on blotter papers using distilled water or full strength Hoagland's solution (Hoagland 1938) as the growth media. Ten lots of twenty seeds were placed in each plexiglass box. The experimental design was a completely randomized design (CRD) with three treatments and four replications. The treatments included seeds germinating in the light with distilled water, seeds germinating in the light with Hoagland's solution, and seeds germinating in the dark with water. Experiments were conducted and repeated in a growth chamber where radiation was maintained at 350 $\mu$mol $m^{-2}$ $s^{-1}$ of PPFD at the top of the boxes with a 16-h photoperiod. Day and night temperatures were 30 C and 20 C, respectively, and day and night relative humidities were maintained at 65 and 85%. For the dark treatment, the plexiglass boxes were covered.

Distilled water and Hoagland's solution were maintained at a depth of 2 cm in the plexiglass boxes. Solutions moved to the seeds by capillary action. Distilled water was added to compensate the water loss through evaporation every 2 d, and Hoagland's solution was replaced once 7 d after the experiment began. One seed lot per replication was sampled from each treatment every 1 or 2 d to evaluate endosperm weight change until no change was observed. At each sampling, ten woolly cupgrass seedlings from each replication were

arbitrarily selected and separated into shoots, roots, and endosperm. Plant parts were dried at 80 C for 48 h and weighed. Data were subjected to analysis of variance with the Statistical Analysis System (SAS). Means were separated by least significant difference at the 5% level of significance.

**Effect of germination depth on woolly cupgrass seed reserve utilization and tiller production**

The experiment was conducted in the greenhouse and radiation was supplemented with 1000 watt high pressure sodium lamps providing a total of 450 to 550 $u$mol $m^{-2}s^{-1}$ of PPFD at the canopy level with a 16-h photoperiod. Radiation could increase up to 850 $u$mol $m^{-2}s^{-1}$ of PPFD at midday. Day and night temperatures were 28 C and 20 C, respectively. Woolly cupgrass seedlings were grown in 15-cm-diam. ceramic pots with potting soil mixture of soil:perlite:peat (2:1:2 by wt.). Seeds were buried 1, 3, 5, 7, or 9 cm deep and pots were thinned to 10 plants per pot after emergence. Plants were sampled at the date of emergence (DOE), at 15 d after planting (DAP), and at 14 d after emergence (DAE). At each sampling date, four pots were arbitrarily selected. The experimental design was a completely randomized design (CRD). Ten plants from each replication were separated into endosperm, mesocotyl, and shoots, dried at 80 C for 48 h, and weighed. Tiller number was also recorded. Data were subjected to analysis of variance with the Statistical Analysis System (SAS). Means were separated by least significance difference at the 5% level of significance.

## RESULTS AND DISCUSSION

### Effect of light and nutrients on endosperm utilization by woolly cupgrass

Endosperms lost weight rapidly until d 6 at which time weight loss slowed (Figure 1). Endosperm weight decline stopped after 8 d for germination in light with Hoagland's solution, and 10 d for germination in light or in dark with water. The endosperm was considered depleted when no further endosperm weight loss occurred (Murray and Cooper 1967). There was no significant difference over time for endosperm consumption comparing germination in light with water and in dark with water. These results were consistent with the results reported by Cooper and MacDonald (1970) in corn (Zea mays L.).

Significant differences in woolly cupgrass endosperm consumption were observed comparing germination in light with Hoagland's solution and germination in dark with water. Woolly cupgrass seeds that germinated in the light with Hoagland's solution consumed endosperm faster than in the dark with water (Figure 1). Shoot growth was significantly faster in light with Hoagland's solution than in other treatments (Figure 2). Nutrients obviously played an important role in seedling growth. From d 3 to d 6, germination in the light with Hoagland's solution resulted in higher shoot dry weight than the other treatments. No significant difference in shoot dry weight comparing germination in the light with water and in the dark with water was observed.

After d 8, there were significant differences in shoot dry weights among treatments (Figure 2). Cooper and MacDonald (1970) reported that the green leaf prior to 12 d had little effect on growth of corn seedlings or rate of endosperm utilization. Although light is an essential component of photosynthesis, germination in light without a nutrient supply limited

the seedling growth. This might explain why there was no difference in shoot growth between germination in light with water and in dark with water.

Root growth had the same trend as shoot growth among treatments (Figure 3). Root growth was limited in dark with water, presumably because the endosperm was the only energy source. Woolly cupgrass seeds germinating and growing in light with Hoagland's solution had more shoot and root growth and also created a larger sink for endosperm utilization at the early growth stage than seed germinating in water, regardless of light treatment. Woolly cupgrass seed endosperm was depleted 8 to 10 d after germination, depending on treatment.

**Effect of germination depth on woolly cupgrass seed reserve utilization and tiller production**

Woolly cupgrass germination depth was measured from the seed to the top of mesocotyl, and was close to the planting depth (Table 1). Woolly cupgrass seed planted 7 to 9 cm had a significantly lower emergence and took more days to emerge, when compared to germination from the depths of 1 to 5 cm (Table 1). Similar results have been reported in many other crops (Gan et al. 1992; Murphy and Arny 1939; O'Connor and Gusta 1994). Seeds germinating deeper in the soil consumed more seed reserves stored in the endosperm. Mesocotyl dry weight increased while endosperm dry weight decreased with increasing germination depths (Figure 4), proving that seedlings expended more energy emerging when deeper in the soil. Plants germinating from a 1 cm depth used less energy to emerge than others, and had more residual endosperm weight (Figure 4). Endosperm weights were not

different regardless of germination depth 15 DAP which was similar to values at d 10 or d 12 in Figure 1. This suggested that seed reserves were used up by 15 DAP regardless of depths (Figure 4). There was no difference in endosperm dry weight between emergence and 15 DAP for plants germinating from 9 cm (Figure 4). It was believed that the endosperm was depleted at the time of emergence for plants germinating from 9 cm.

Peterson et al. (1989) reported that excision of the endosperm in wheat delayed the first two phyllochrons, prevented outgrowth of the coleoptilar tiller, and slightly delayed the emergence of the next two tillers. In our study, differences in residual endosperm dry weight at the time of emergence caused by different germination depths affected seedling growth and tiller production. At 15 DAP, plants germinating from 1 and 3 cm had more tillers than those from 5 to 9 cm (Table 2). The difference was mostly observed in tiller 2 production. Tiller 1 emergence decreased for 9 cm germination. At 14 DAE, tiller production of plants germinating from different depths was not significantly different except for plants germinating from 9 cm (Table 3).

Deeper planting significantly decreased shoot dry weight at 15 DAP (Table 4). The difference was caused, in part, by the shorter time available for photosynthesis associated with late emergence (Huang and Taylor 1993). At 14 DAE, there were no significant differences in shoot dry weight among germination depths except for germination from 3 cm which had significantly higher shoot dry weight than other treatments. Bremner et al. (1963) and Peterson et al. (1989) reported that the development of leaves on the seedling main culm occurred in two sequential phases. Leaf development rate (LDR) for the initial phase was affected by seed size and endosperm excision treatments. The LDR for the final phase was

similar for seed size and endosperm excision treatments. Neither seed size nor endosperm excision affected LDR once the seedling became independent of the seed reserves. At 14 DAE, similar shoot dry weights were observed in all germination depths probably because seedlings had the same period of time for photosynthesis.

Woolly cupgrass endosperms were depleted 8 to 10 d after germination. Seedlings grew faster in light with Hoagland's solution than in dark with water, thus creating a larger sink, and consuming endosperm faster. The woolly cupgrass first tiller usually appeared 10 to12 DAP, therefore woolly cupgrass endosperm depletion occurred before the emergence of the first tiller. It is not likely that the endosperm supported the new tiller growth and emergence. The germination depth study also supported this conclusion. Other factors which might contribute to new tiller emergence need to be examined. Deeper planting increased emergence time, reduced seedling dry weight and tiller production. Seedlings emerging late would have a shorter time for photosynthesis, and therefore would produce less dry matter in the early stage.

## SOURCES OF MATERIALS

[1] Hoagland's No.2. basal salt mixture. Sigma Chem. Co. St. Louis, MO 63178.

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Table 1. Effect of planting depth on woolly cupgrass germination and emergence.

| Planting depth | Germination depth | Days to emergence | Emergence |
|---|---|---|---|
| cm | cm | | % |
| 1 | 1.3±0.06[a] | 4 | 100 a[b] |
| 3 | 3.2±0.19 | 5 | 100 a |
| 5 | 4.7±0.55 | 5 | 100 a |
| 7 | 6.8±0.12 | 6 | 88 b |
| 9 | 8.8±0.41 | 8 | 58 c |

[a] Values are means ± standard deviations of the means.

[b] Means in same column followed by the same letter are not significantly different at P=0.05, according to the LSD test.

Table 2. Effect of planting depth on woolly cupgrass tiller production 15 days after planting.

| Planting depth | Main stem | Tiller 1 | Tiller 2 |
|---|---|---|---|
| cm | | %[a] | |
| 1 | 100 a[b] | 42 a | 83 a |
| 3 | 100 a | 20 ab | 73 a |
| 5 | 100 a | 18 ab | 28 b |
| 7 | 84 a | 28 ab | 8 b |
| 9 | 58 b | 5 b | 0 c |

[a] % was calculated based on 10 plants for each replication.

[b] Means in same column followed by the same letter are not significantly different at P=0.05, according to the LSD test.

Table 3. Effect of planting depth on woolly cupgrass tiller production 14 days after emergence.

| Planting depth | Main stem | Tiller 1 | Tiller 2 | Tiller 3 |
|---|---|---|---|---|
| cm | | | %[a] | |
| 1 | 100 a[b] | 25 b | 93 a | 0 b |
| 3 | 100 a | 60 a | 95 a | 15 a |
| 5 | 100 a | 40 ab | 95 a | 3 b |
| 7 | 100 a | 60 a | 80 b | 0 b |
| 9 | 100 a | 20 b | 58 c | 15 a |

[a] % was calculated based on 10 plants for each replication.

[b] Means in same column followed by the same letter are not significantly different at P=0.05, according to the LSD test.

Table 4. Effect of planting depth on woolly cupgrass seedling shoot dry weight.

| Planting depth | 15 DAP[a] | 14 DAE[b] |
|---|---|---|
| cm | g[c] | |
| 1 | 0.60 a[d] | 0.91 ab |
| 3 | 0.57 a | 1.14 a |
| 5 | 0.35 b | 0.86 b |
| 7 | 0.26 b | 0.86 b |
| 9 | 0.12 c | 1.08 ab |

[a] DAP: days after planting.
[b] DAE: days after emergence.
[c] The average of 4 10-plant replications.
[d] Means in same column followed by the same letter are not significantly different at P=0.05, according to the LSD test.

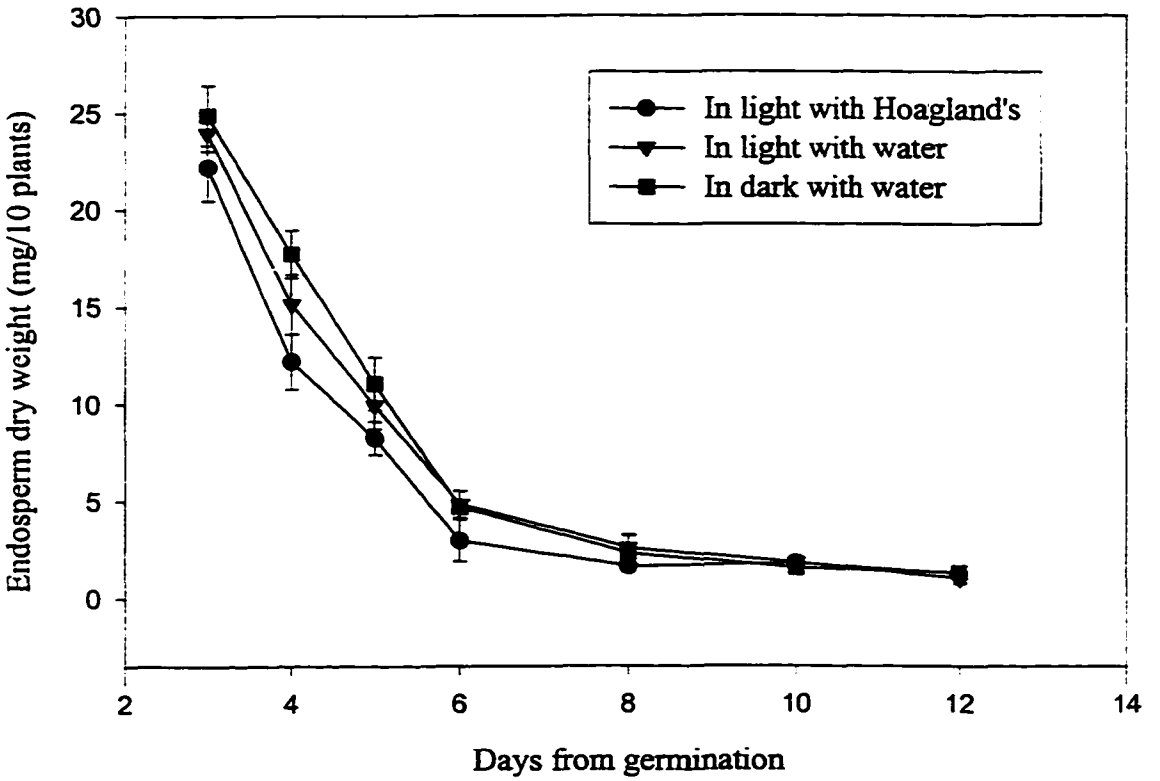
## LIST OF CAPTIONS

Figure 1. Effect of growth medium and light on woolly cupgrass endosperm utilization. Bars represent standard deviation of the mean. Regression equations for endosperm dry weight over time are $y = 43.89 - 9.51\ x + 0.51\ x^2$, $R^2 = 0.91$, $y = 46.68 - 9.64\ x + 0.50\ x^2$, $R^2 = 0.94$, and $y = 50.84 - 10.55\ x + 0.54\ x^2$, $R^2 = 0.96$ for germination in light with Hoagland's solution, in light with water, and in dark with water.

Figure 2. Effect of growth medium and light on woolly cupgrass seedling shoot growth. Bars represent standard deviation of the mean. Regression equations for shoot dry weight over time are $y = 27.57 - 13.84\ x + 2.30\ x^2$, $R^2 = 0.99$, $y = -18.82 + 6.57\ x$, $R^2 = 0.99$, and $y = -14.90 + 6.88\ x - 0.34\ x^2$, $R^2 = 0.92$ for germination in light with Hoagland's solution, in light with water, and in dark with water.

Figure 3. Effect of growth medium and light on woolly cupgrass seedling root growth. Bars represent standard deviation of the mean. Regression equations for root dry weight over time are $y = 28.31 - 10.85\ x + 1.21\ x^2$, $R^2 = 0.97$, $y = -3.10 + 2.07\ x$, $R^2 = 0.99$, and $y = 4.56$ for germination in light with Hoagland's solution, in light with water, and in dark with water.

Figure 4. Effects of planting depth on woolly cupgrass endosperm dry weight at emergence, 15 days after planting (DAP), and mesocotyl dry weight at emergence. Bars represent standard deviation of the mean. Regression equation for endosperm dry weight at emergence, $y = 21.20 - 2.77\ x + 0.07\ x^2$, $R^2$=0.84; for mesocotyl, $y = 2.85 + 1.79\ x$, $R^2 = 0.99$; for endosperm dry weight 15 days after planting, $y = 1.5$.




Liu et al.
Figure 1

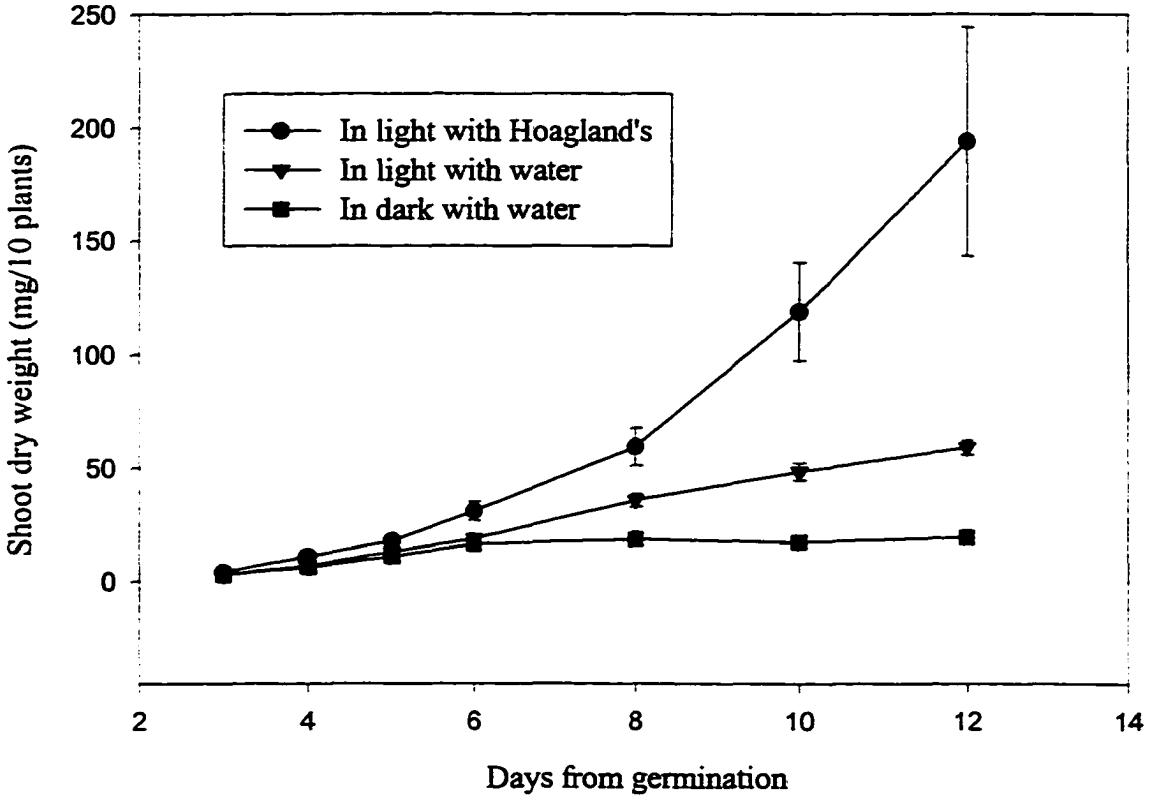



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Figure 2

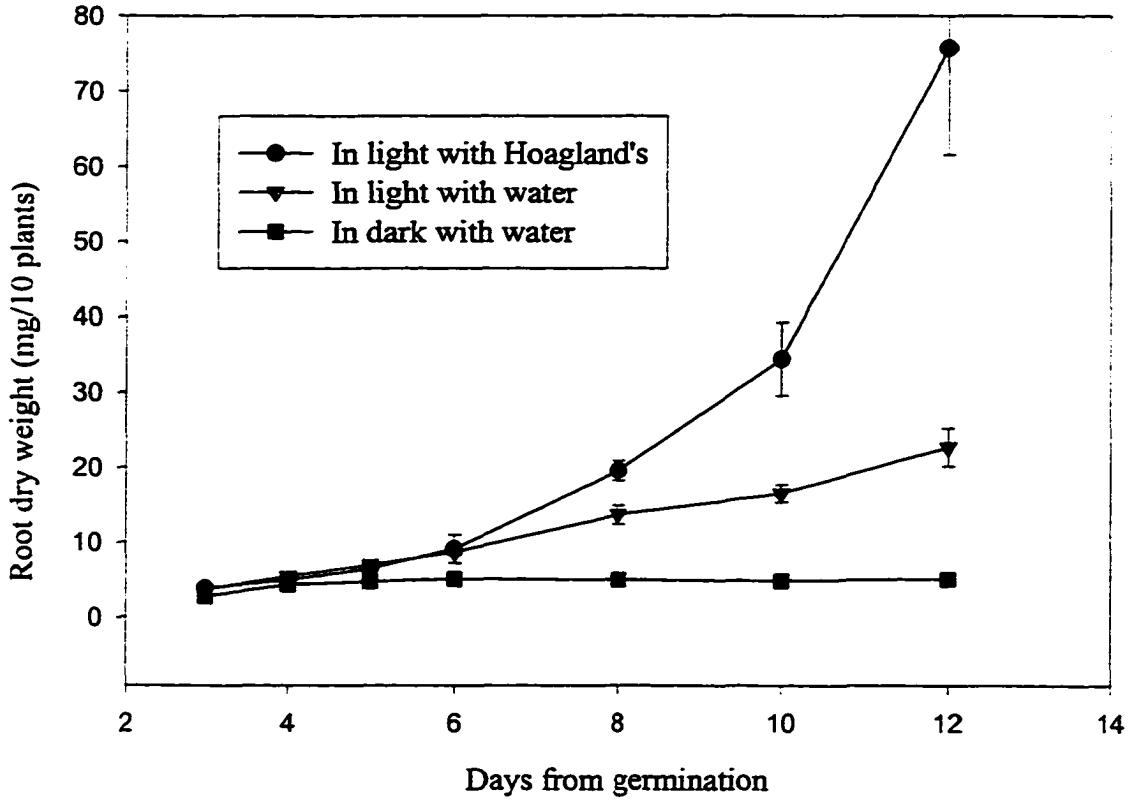


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Figure 3

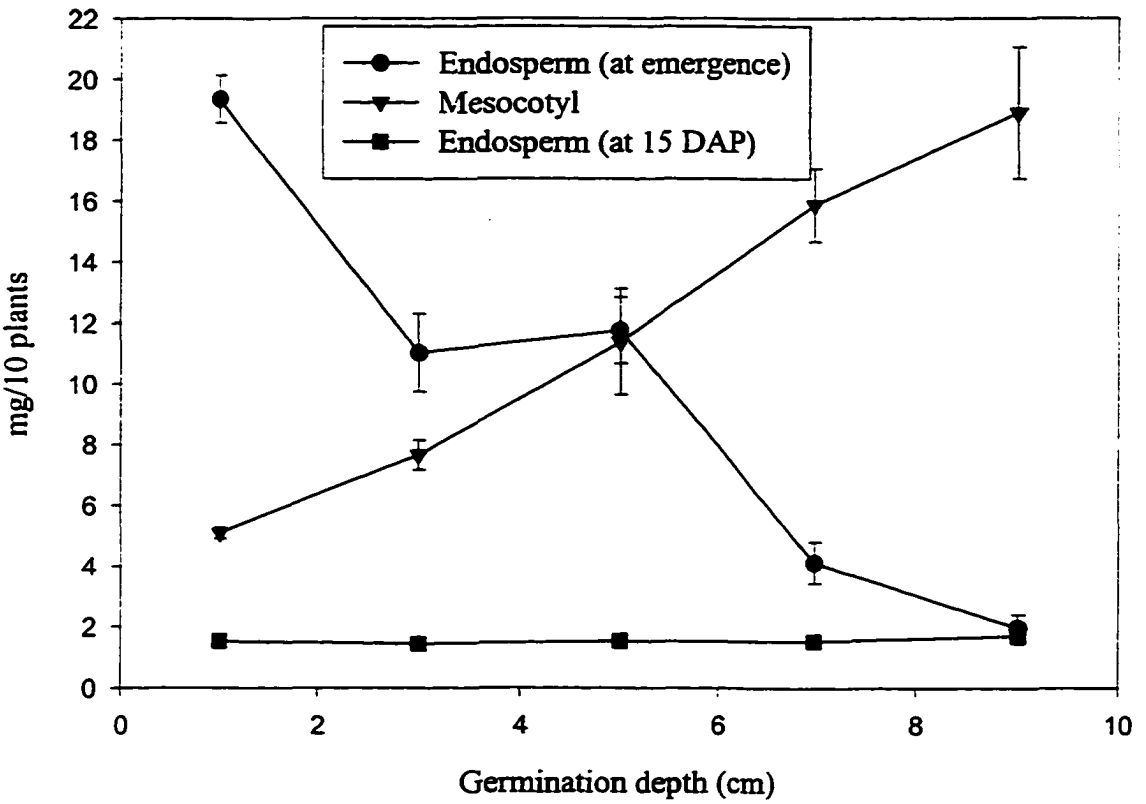


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Figure 4

# EFFECTS OF NICOSULFURON RATES AND MAIN STEM EXCISION ON WOOLLY CUPGRASS (Eriochloa villosa) TILLER EMERGENCE

A paper to be submitted to Weed Science

Ming-Chung Liu and Micheal D. K. Owen

## ABSTRACT

Main stem excision allowed tillers to develop quickly following nicosulfuron application. When treated with 35 g ai $ha^{-1}$ nicosulfuron and keeping the main stem intact, 1.4 and 3.9 tillers per woolly cupgrass plant were produced 10 and 21 days after herbicide treatment (DAT), respectively. No tillers were produced for 70 and 140 g ai $ha^{-1}$ nicosulfuron with an intact main stem 10 DAT. Only 0.9 tillers per plant were produced with the 70 g ai $ha^{-1}$ treatment 21 DAT. Tiller growth was affected by the nicosulfuron rate. The length of tiller two and tiller three were 24.8 mm and 19.5 mm for 35 g ai $ha^{-1}$ treatment; 15.6 mm and 14 mm in 70 g ai $ha^{-1}$ treatment, and 8.4 mm and 3.9 mm in 140 g ai $ha^{-1}$ treatment 14 DAT. Tillers were still growing inside the leaf sheath when the main stem was inhibited. However, most of the tillers did not emerge due to the physical constraint imposed by the leaf sheath. The recommended nicosulfuron rate, 35 g ai $ha^{-1}$, inhibited the growth of the main stem, yet did not completely inhibit the tiller growth, resulting in prolific tillering.

**Nomenclature:** Nicosulfuron, 2-[[[[(4,6-dimethoxy-2-pyrimidinyl)amino]carbonyl] amino]sulfonyl]-*N*,*N*-dimethyl-3-pyridicarboxamide; woolly cupgrass, Eriochloa villosa

(Thunb.) Kunth. # ERBVI.

**Additional index words:** Leaf sheath constraint, main stem excision, tiller emergence, ERBVI.

## INTRODUCTION

Many postemergence herbicides cause growth abnormalities in both crops and weeds. Sublethal doses of glyphosate (*N*-(phosphonomethyl)glycine) applied to the foliage of quackgrass (Agropyron repens) stopped the growth of shoots and caused prolific tillering (Caseley 1972). Under normal growth conditions, basal buds in sorghum (Sorghum bicolor) do not develop; however, application of glyphosate stimulated basal bud development (Baur et al. 1977). Chao et al. (1993) reported that foliar application of imazamethabenz ((±)-2-[4, 5-dihydro-4-methyl-4-(1-methylethyl)-5-oxo-1 *H*-imidazol-2-yl]-4(and 5)-methylbenzoic acid (3:2)) at sublethal doses to wild oat (Avena fatua) greatly inhibited the growth of the main shoot but increased tillering. Postemergence application of fluazifop ((±)-2-[4-[[5-(trifluoromethyl)-2-pyridinyl]oxy]phenoxy]propanic acid) caused tiller regrowth in goosegrass (Eleusine indica), large crabgrass (Digitaria sanguinalis), and giant foxtail (Setaria faberi) (Derr et al. 1985). Stunted main stems and prolific tillering are common responses of grasses to sublethal or recommended rates of many herbicides. Tiller regrowth was herbicide rate and species dependent (Derr et al. 1985).

Increased tillering resulted from resumed growth of tillers and the increased initiation of tiller buds. Such enhanced tillering mainly resulted from the release of apical dominance due to herbicidal inhibition of the main stem growth (Chao et al. 1993). Chao et al. (1994)

further demonstrated that main shoot apical dominance limited imazamethabenz translocation to lateral shoots, rendering tillers less susceptible to growth inhibition by the herbicide. The release of apical dominance has also been demonstrated with glyphosate on Cynodon dactylon (Fernandez and Bayer 1977), wheat (Triticum aestivum) and sorghum (Baur et al. 1977). Shieh et al. (1993) reported that the sink activity of various buds along old rhizomes of quackgrass increased, thus competing strongly with other nearby buds, which consequently received little assimilates and glyphosate. When the more active buds were killed by glyphosate, those with a sublethal dose of glyphosate likely would be released from dormancy.

In previous studies, woolly cupgrass was reported to survive a recommended rate of nicosulfuron by initiating new tillers (Pullins 1995). The emergence of woolly cupgrass tillers following nicosulfuron application has not been attributed to sublethal herbicide effects or determined if other factors were involved. An 11-day-old woolly cupgrass seedling had a total of 9 tiller buds initiated when the first tiller emerged (Liu and Owen, unpublished data). We hypothesized that apical dominance was removed when the main stem was stunted by nicosulfuron, thus allowing existing tillers to grow. However, the release of apical dominance could not fully explain the emergence of tillers. Other factors were restricting tiller emergence.

The early growth of tiller buds occurred within a tightly packed cavity in the leaf axil (Williams and Langer, unpublished). Williams et al. (1975) reported that physical constraint imposed on tiller buds by surrounding leaf sheaths, in combination with environmental factors, might determine the tillering pattern of wheat. Williams and Metcalf (1975) also

found that the application of additional physical constraints on wheat seedlings prevented the emergence of all tillers, yet the removal of the coleoptile allowed the coleoptilar tiller to grow vigorously. Robertson et al. (1989) reported that the scale leaves of quackgrass rhizomes inhibited the early development of axillary buds. Denudation or simply pulling the scale leaves back so that they no longer contacted the axillary bud promoted sprouting but did not support the subsequent elongation of buds as effectively as decapitation. The role of leaf sheath in restricting woolly cupgrass tiller growth has not been documented.

Our objectives were to (a) determine whether the woolly cupgrass tiller emergence was caused by the release of apical dominance, (b) determine the effects of nicosulfuron rate on tiller emergence and (c) characterize the constraint imposed by the leaf sheath on tiller emergence of woolly cupgrass following nicosulfuron application.

## MATERIALS AND METHODS

Woolly cupgrass seeds used for the following experiment were collected from native woolly cupgrass populations in a corn field at Ogden, Iowa, in September 1995. Seeds were air-dried and stored at 4 C until used.

The experiments were conducted in a growth chamber. Radiation was maintained at 350 $\mu$mol $m^{-2}$ $s^{-1}$ of PPFD at the canopy level with a 16-h photoperiod. Day and night temperatures were 30 C and 20 C, respectively, and day and night relative humidities were maintained at 65 and 85%. Woolly cupgrass seeds were planted in 10-cm-diam. pots with potting soil mixture of soil:perlite:peat (2:1:2 by wt.), and pots were thinned to 5 plants per pot 5 days after planting (DAP). Twelve DAP, when woolly cupgrass seedlings were 4 cm

with 3.5 leaves, nicosulfuron treatments of 35, 70, 140 g ai $ha^{-1}$ and an untreated control were applied and each treatment was replicated 8 times. Nicosulfuron was applied with a spray chamber delivering 234 L $ha^{-1}$ at 207 kPa. Main stem excision treatments were applied 96 hours after herbicide treatment (HAT). Pots from each herbicide treatment were randomly assigned one of two excision treatments. In the first treatment, the main stem of woolly cupgrass was excised approximately 0.5 to 1 cm above soil surface, and in the second treatment, the main stem remained intact. The experiment was a factorial design with four replications where herbicide was the main treatment. Tiller emergence or re-growth was photographed every week for three weeks, tiller numbers were recorded at 10 and 21 DAT, and plant height and fresh weight were recorded 24 DAT. Extra woolly cupgrass plants were planted and sprayed at the same time as the experiment described above to compare the tiller growth following nicosulfuron application. Four pots were randomly selected from every herbicide treatment and 20 plants from four pots were used to calculate tiller growth. The length of leaf sheath and the length of tiller within the leaf sheath were measured weekly for three weeks. All experiments were repeated and data were subjected to analysis of variance using the Statistical Analysis System (SAS). Means were separated by least significant difference at the 5% level of significance when appropriate.

## RESULTS AND DISCUSSION

Woolly cupgrass growth and development were rapidly inhibited by nicosulfuron. The Haun stage (number of leaves on the main stem) and the plant height of the untreated control plants were significantly greater than all nicosulfuron treatments (Table 1). There were no

significant differences among nicosulfuron rates. Haun stage and plant height for treated plants did not change much after nicosulfuron application. The commercially recommended nicosulfuron rate, 35 g ai $ha^{-1}$, successfully inhibited woolly cupgrass seedling growth. Lycan and Hart (1998) demonstrated that nicosulfuron absorption by woolly cupgrass reached 52% 12 HAT, and had not significantly increased at 72 HAT. Our preliminary experiment showed that nicosulfuron translocation out of treated leaf reached the maximum 120 HAT and 75% of the maximum translocation was reached 96 HAT (data not shown). Ninety-six hours of retention was long enough for most nicosulfuron to be absorbed and translocated to the site of action.

Tillers quickly emerged in the untreated control and the 35 g ai $ha^{-1}$ nicosulfuron treatment after the main stems were excised. The emergence of tillers for the 70 and 140 g ai $ha^{-1}$ treatments was delayed (Figure 1). In the treatments where the main stem was not excised, plants treated with 35 g ai $ha^{-1}$ nicosulfuron demonstrated prolific tillering while the plants from the 70 and 140 g ai $ha^{-1}$ treatments never tillered during the experiment. The main stems of the 70 and 140 g ai $ha^{-1}$ treatments were necrotic, and plants eventually died.

Grasses have been reported to have prolific tillering after the application of sublethal or recommended rates of herbicides (Caseley 1972; Chao et al. 1993; Derr et al. 1985). Chao et al. (1994) demonstrated that the apical dominance imposed by the main shoot limited herbicide translocation to lateral shoots, rendering tillers less susceptible to growth inhibition by the herbicide. In the treatment of 35 g ai $ha^{-1}$ nicosulfuron with main stem intact, tiller buds might have received less herbicide, and thus tiller two (T2) and three (T3) resumed growth and emerged. We assumed that more herbicide was translocated to the tiller buds in

the 70 and 140 g ai $ha^{-1}$ treatments than in the 35 g ai $ha^{-1}$ treatment. Herbicide translocation to the tiller buds was increased due to the release of apical dominance resulting from the herbicide effect on the main stem, thus successfully inhibiting tiller growth and emergence.

Removing the main stem allowed tillers to emerge quickly (Table 2). Tiller numbers significantly increased when the main stem was excised. In the treatment of 35 g ai $ha^{-1}$ nicosulfuron with main stem intact, 1.4 tillers per plant were produced, yet in the treatments of 70 and 140 g ai $ha^{-1}$ with main stem intact, no tillers were produced 10 DAT. Nicosulfuron at 35 g ai $ha^{-1}$ allowed more tillers to be produced in the main stem intact than with the main stem excised when evaluated 21 DAT. Few or no tillers were produced in the 70 and 140 g ai $ha^{-1}$ nicosulfuron treatments with the main stem intact. Nicosulfuron at 140 g ai $ha^{-1}$ significantly reduced tiller numbers even when the main stem was excised.

Woolly cupgrass tiller height and fresh weight were significantly reduced by nicosulfuron when compared with the untreated control (Table 3). Tiller height and fresh weight decreased significantly with increased nicosulfuron rates. This agreed with our observations that 35 g ai $ha^{-1}$ nicosulfuron inhibited woolly cupgrass growth, yet did not completely inhibit tiller growth.

Niklas (1998) demonstrated that the leaf sheath of Arundinaria tecta acted as an external cylindrical brace and contributed 33% of the overall bending stiffness and 43% of the overall torsional stiffness of stem segments. Williams et al. (1975) reported that the physical constraint on tiller buds by the surrounding leaf sheaths, in combination with environmental factors, might determine the tillering pattern of wheat. Scale leaves inhibited the development of axillary buds of quackgrass rhizomes and removal of the scale leaves

promoted an initial burst of bud growth regardless of the apex (Robertson et al. 1989). The physical constraint of the leaf sheath, therefore, was considered as a factor restricting tiller emergence.

The excision of the woolly cupgrass main stem was designed to remove the constraint presumably imposed by the leaf sheath. T2 and T3 tillers quickly emerged when the main stem was excised compared to the treatment where the main stem remained intact. Removing the main stem may have removed the apical dominance imposed by the main stem, thus allowing tillers to grow and emerge. However, there was no tiller emergence where woolly cupgrass was treated with nicosulfuron and the main stem kept intact. The apical dominance was considered removed due to the inhibition or cessation of the main stem growth attributable to the herbicide treatment (Chao et al. 1993). Tiller emergence where the main stem was excised could not be attributed to the release of apical dominance alone. In an experiment where L2 and L3 were excised from the base of the leaf sheath and L4 and the shoot apex remained intact, T2 and T3 also emerged, but slower than when main stem was excised. This response suggested that tiller growth was still affected by the apical dominance imposed by the shoot apex and un-expanded leaves.

When the tiller length within the leaf sheath was examined, the tillers were still growing inside the leaf sheath even though the growth of main stem was inhibited (Table 4). The growth of the first and the second leaf sheaths were completed at the time of nicosulfuron application, and the length of the third leaf sheath increased little after application (data not shown). Tiller growth in all nicosulfuron treatments was significantly slower than that of the untreated control. Tiller 1 had already begun to elongate at the time of application and

emerged from the leaf sheath even though elongation rate was negatively affected by nicosulfuron. No tiller 2 emerged from the leaf sheath 7 and 14 DAT except that the leaf tip of tiller 2 began to emerge in the 35 g ai $ha^{-1}$ treatment 14 DAT. Tiller 2 growth increased with time but was significantly reduced by 70 and 140 g ai $ha^{-1}$ nicosulfuron compared to the 35 g ai $ha^{-1}$ treatment. Tiller 3 was small at the time of application and 7 DAT, however the growth significantly increased by 14 DAT. The growth of tiller 3 was significantly reduced by 140 g ai $ha^{-1}$ nicosulfuron when compared to the 35 and 70 g ai $ha^{-1}$ treatments.

In conclusion, tiller growth following nicosulfuron application was affected by the presence of the main stem. The main stem presumably was the source of apical dominance and the leaf sheath was a physical constraint restricting the growth of tiller buds. Removing the main stem released apical dominance and the physical constraint, thus allowing tillers to emerge and grow quickly. Removing the apical dominance by the application of nicosulfuron alone did not release tillers from the physical constraint imposed by the leaf sheaths. Tillers grew inside the leaf sheaths, yet typically were not able to emerge. Tiller growth was also affected by the rate of nicosulfuron. Nicosulfuron at the recommended rate of 35 g ai $ha^{-1}$ inhibited the growth of the main stem, yet did not completely inhibit tiller growth, resulting in prolific tillering.

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Table 1. Effect of nicosulfuron on Haun stage and height of woolly cupgrass.

| Nicosulfuron<br>__ g ai $ha^{-1}$ __ | At application | 1 WAT[a] | 2 WAT |
|---|---|---|---|
| | | Haun stage | |
| 35 | | 3.8 b[d] | 3.9 b |
| 70 | | 3.7 b | 3.9 b |
| 140 | | 3.8 b | 3.9 b |
| Untreated control | 3.5±0.3[b] | 5.4 a | 6.7 a |
| | | Height[c] | |
| | | cm | |
| 35 | | 4.2 b | 4.4 b |
| 70 | | 4.0 b | 4.3 b |
| 140 | | 4.6 b | 4.3 b |
| Untreated control | 4.0±0.7 | 8.5 a | 12.6 a |

[a] WAT: weeks after treatment.
[b] Values were mean ± standard deviation and were calculated from 20 plants.
[c] Plant height was measured from the soil surface to the uppermost leaf collar.
[d] Means within columns followed by the same letter were not significantly different at 5% level.

Table 2. Effect of nicosulfuron and main stem excision on woolly cupgrass tiller emergence.

| Nicosulfuron g ai $ha^{-1}$ | Main stem | Tillers $plant^{-1}$ | |
|---|---|---|---|
| | | 10 DAT[a] | 21 DAT |
| 35 | excised | 2.9 a[b] | 2.4 bc |
| 35 | intact | 1.4 c | 3.9 a |
| 70 | excised | 2.3 ab | 3.5 ab |
| 70 | intact | 0 d | 0.9 de |
| 140 | excised | 1.6 bc | 1.3 cd |
| 140 | intact | 0 d | 0 e |

[a] DAT: days after treatment.
[b] Means within columns followed by the same letter were not significantly different at 5% level.

Table 3. Effect of nicosulfuron and main stem excision on woolly cupgrass height and weight.

| Nicosulfuron | Main stem | Height | Fresh weight |
|---|---|---|---|
| g ai $ha^{-1}$ | | cm | g $plant^{-1}$ |
| 35 | intact | 21.3 b | 2.7 b |
| 35 | excised | 21.2 b | 1.8 c |
| 70 | excised | 12.6 c | 0.7 d |
| 140 | excised | 6.4 c | 0.1 d |
| Untreated control | intact | 47.0 a | 4.7 a |

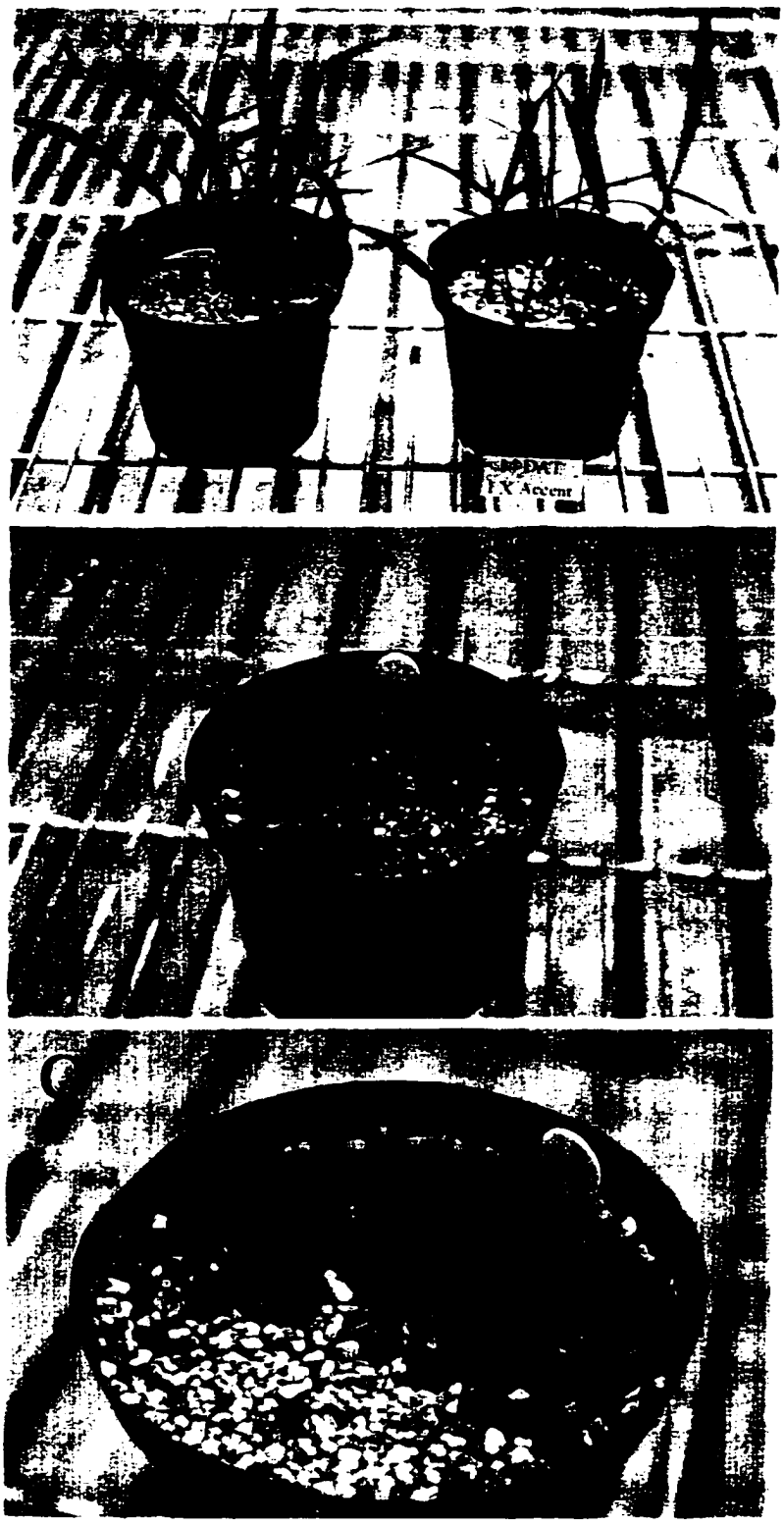
Data were taken at 24 days after treatment. Plants were dead in 70 and 140 g nicosulfuron treatments with the main stem intact, thus data was not included. Means within columns followed by the same letter were not significantly different at 5% level.

Table 4. Effect of nicosulfuron on woolly cupgrass tiller growth.

| Nicosulfuron<br>g ai $ha^{-1}$ | Length of tiller<br>mm | | |
|---|---|---|---|
| | At application | 1 WAT[a] | 2 WAT |
| | | Tiller 1 | |
| 35 | | 14.0 b[c] | 17.8 a |
| 70 | | 15.2 b | 12.2 a |
| 140 | | 14.1 b | 14.3 a |
| Untreated control | 6.3±4.7[b] | 25.3 a | -[d] |
| | | Tiller 2 | |
| 35 | | 6.8 b | 24.8 a |
| 70 | | 5.6 b | 15.6 b |
| 140 | | 6.9 b | 8.4 b |
| Untreated control | 2.9±1.7 | 40.1 a | - |
| | | Tiller 3 | |
| 35 | | < 1 | 19.5 a |
| 70 | | < 1 | 14.0 a |
| 140 | | < 1 | 3.9 b |
| Untreated control | < 1 | 32.4 a | - |

[a] WAT: weeks after treatment.
[b] Values were mean ± standard deviation and were calculated from 20 plants.
[c] Means within columns followed by the same letter were not significantly different at 5% level.
[d] Tillers longer than leaf sheaths 2 WAT were not included.

Figure 1. Effect of nicosulfuron on woolly cupgrass tiller emergence 14 days after application following the excision of main stem. A. Right. Woolly cupgrass plants showed much more healthy tillers at 35 g ai $ha^{-1}$ nicosulfuron compared to 70 and 140 g ai $ha^{-1}$ rates; Left. Woolly cupgrass plants showed prolific tillering at 35 g ai $ha^{-1}$ rate. B and C. Woolly cupgrass tillers were negatively affected by 70 and 140 g ai $ha^{-1}$ nicosulfuron even though main stems were excised.



Liu et al.
Figure 1

# WOOLLY CUPGRASS (Eriochloa villosa) POPULATION RESPONSE TO TILLAGE AND HERBICIDE USE

A paper to be submitted to Weed Science

Ming-Chung Liu and Micheal D. K Owen

## ABSTRACT

Weed seeds germinate sporadically in time and space. Late woolly cupgrass germination is assumed to be partly responsible for the unsatisfactory control in soybean and corn, particularly where herbicides are the only management strategy. The objective of this study was to investigate the effects of tillage and herbicides on the population dynamics and germination patterns of woolly cupgrass. Two experiments were established at the Armstrong Research Station near Lewis, Iowa on a natural woolly cupgrass infestation and conducted for three consecutive years. Five herbicide treatments and two tillage regimes were included in the experiments. Conventional tillage significantly decreased woolly cupgrass populations at the second germination event in 1994 and 1995 in experiment one, but not in experiment two. Generally, woolly cupgrass populations decreased significantly after the first germination event. There were differences in populations among herbicide treatments; however, these differences were not as important as the differences between the first germination event and subsequent germination events. Germination depth of woolly cupgrass in conventional tillage was significantly deeper than in no-tillage from the second to

the fourth 1994 germination events in experiment one. In 1995, tillage had a significant effect on the germination depth of the second event and the germination depth in conventional tillage was deeper than in no-tillage. In experiment two, no significant effect of tillage was observed. Germination depth increased for later germination events; however, differences among herbicides were not significant. When seed production was eliminated, the woolly cupgrass seedbank was depleted in three years.

**Nomenclature:** Acetochlor, (2-chloro-*N*-(ethoxymethyl)-*N*-(2-ethyl-6-methylphenyl) acetamide); dimethenamid, (2-chloro-*N*-(1-methyl-2-methoxy)ethyl)-*N*-,4-dimethylthien-3-yl)acetamide); metolachlor, (2-chloro-*N*-(2-ethyl-6-methylphenyl)-*N*-(2-methoxy-1-methylethyl)acetamide); pendimethalin, (*N*-(1-ethylpropyl)-3,4-dimethyl-2,6-dinitrobenzenamine); glyphosate, (*N*-(phosphonomethyl)glycine); woolly cupgrass, Eriochloa villosa (Thunb.) Kunth. #ERBVI.

**Additional index words:** Conventional tillage, germination depth, no-tillage, preemergence herbicides, weed population, ERBVI.

## INTRODUCTION

Tillage affects weed species composition and depth of seed distribution in soil (Teasdale et al. 1991; Wrucke and Arnold 1985; Yenish et al. 1992). Changes in tillage can have a significant effect on weed populations and thus weed control (Ball and Miller 1990; Egley and Williams 1990). Tillage affects the soil seedbank by moving weed seeds buried deep in the soil nearer to the soil surface and seeds from the soil surface deeper in the soil thus increasing seed longevity and potentially prolonging the life of the seedbank. However,

greater weed emergence in cultivated, compared to undisturbed plots, resulted in a faster decline of the soil seed reserves in the cultivated plots (Cardina et al. 1991; Egley and Williams 1990). Ogg and Dawson (1984) found that the seedbank response to tillage was species dependent; emergence of four broadleaf weed species was stimulated, three broadleaf species were unaffected, and emergence of one grass species was depressed by tillage. No-tillage systems typically had higher populations of small-seeded annual weeds while tillage systems using moldboard plows had more large-seeded annual weeds. Roberts and Potter (1980) demonstrated that weed emergence was dependent on the timing of cultivation relative to rainfall.

Under a consistent cultivation regime, there was a relationship between the number of viable seeds in the soil and the plants found during the growing season (Roberts and Dawkins 1967). In other studies, the relationship between weed seed populations in the soil and plants growing in the field has been variable and not predictable (Ball and Miller 1989; Wilson et al. 1985). Weed seedling populations may be predicted by multiplying emergence rates by the size of seedbank (Forcella 1992). However, emergence rates vary according to species, year and management.

Seedbank estimates demonstrate great variation (Cardina and Sparrow 1996; Forcella et al. 1992; Wilson et al. 1985). Ball and Miller (1989) reported that for individual species, seed estimates and subsequent weed counts were poorly correlated. The seed density estimation alone was a poor predictor of the weed flora.

Seed persistence in the soil was affected by germination, dormancy mechanisms and resistance to pathogens (Leck et al. 1989). Thompson et al. (1993) reported that weed seeds

long-lived in the soil tended to be small and compact, while shorted-lived seeds were normally larger and either flattened or elongated. When weed seed production was prevented, weed seedbanks were reduced up to 90% within four to six years, but in one year, weeds going to seed restored the soil seedbank to 50% of the original level (Burnside et al. 1986).

Woolly cupgrass is a relatively new weed problem in the Midwest (Strand and Miller 1980). Woolly cupgrass seeds can germinate continuously during the growing season and seedlings from late germination events usually escape herbicide treatment and add to the management problem (Owen 1990). Most herbicide evaluations for woolly cupgrass control emphasize early season control (Owen et al. 1993; Schuh and Harvey 1991). Little information about woolly cupgrass germination responses to tillage is available, and germination response to the residual effects of preemergence herbicide is also lacking. The objectives of this study were to investigate the effects of tillage and preemergence herbicides on woolly cupgrass populations and germination depth.

## MATERIALS AND METHODS

An experiment was established in 1994 at the Armstrong Research Station near Lewis, Iowa in a natural woolly cupgrass infestation. A second experiment was established in 1995 in a nearby corn field. Experiments were repeated at the same locations for three years. The soil was a Marshall (Exira, silty clay loam, mesic Typic Calciaquolls) with a pH of 5.1 and 3.7% organic matter. Corn was planted in a disk tillage system the year previous to the establishment of the experiments. No crop was planted during the experiments since it has

been reported that leaf-canopy could inhibit weed seed germination and our objectives were to investigate the effects of tillage and residual herbicides (Washitani and Saeki 1984).

The experiments were arranged as split plots in a randomized complete block design with three replications. Main plot treatments included no-tillage and conventional tillage systems. The conventional tillage blocks were established with two passes with a disk adjusted to till at a depth of 8 cm in the first year. In the second and the third years, the conventional tillage treatment was completed with a rotovator adjusted to till 8 cm. The no-tillage blocks were not disturbed throughout the duration of the experiment. Plots did not receive any cultivation after herbicide application. Subplot treatments were preemergence herbicide treatments and included acetochlor applied at 1.2 kg ai $ha^{-1}$, dimethenamid at 0.7 kg ai $ha^{-1}$, metolachlor at 1.4 kg ai $ha^{-1}$, pendimethalin at 0.9 kg ai $ha^{-1}$, and an untreated control.

Herbicides were applied on May 17, 1994, June 1, 1995, and May 22, 1996 to the first experiment, and June 1, 1995, May 22, 1996, and June 5, 1997 to the second experiment. Herbicides were applied with a bicycle sprayer delivering 187 L $ha^{-1}$ at 207 kPa. The subplot size was 3 m by 9 m.

Woolly cupgrass populations and germination depths were measured for each germination event. When woolly cupgrass seedlings were at the 3-4 leaf stage, the cohort was considered as a germination event. Populations were measured by using a 30 cm quadrat as a sampling unit. Three areas were arbitrarily sampled in each plot for each germination event. Germination depth was determined by arbitrarily selecting 10 woolly cupgrass plants from each sample area and measuring the mesocotyl length from the seed scar to the soil surface. After the first or the second germination events, woolly cupgrass germination was

sporadic within subplots, and populations were determined from the entire subplot and 10 or 15 plants from each subplot were used to determine germination depth.

Glyphosate (*N*-(phosphonomethyl) glycine) was used to control emerged plants after each sampling. Woolly cupgrass seed production was prevented during the entire experiment period. Experiment one was located on a slope and experiment two located on a hill top. Woolly cupgrass seeds around the plots would not naturally migrate to the plots in experiment two, but some seeds did migrate to the plots in experiment one due to soil erosion. At the last sampling date of the third year, ten soil cores from each subplot were sampled using 3.5 cm-diameter probe to 15 cm depth and soil cores were combined. Woolly cupgrass seeds were counted following an elutriation procedure to investigate final seed density in the soil (Gross 1990).

Precipitation during the experiment periods varied among years (Table 1). Year 1996 had a wet May and June, and year 1997 was slightly drier than 30-year average. However, rainfall did not seem to limit woolly cupgrass germination in our study. Data was subjected to analysis of variance with the Statistical Analysis System (SAS). Means were separated by using a least significant difference test (LSD) at the 0.05 probability level.

## RESULTS AND DISCUSSION

There was no interaction between tillage and herbicide treatments. Thus, tillage effects and herbicide effects were examined separately. There were six woolly cupgrass germination events in 1994, four in 1995, and four in 1996 at the first experiment, and four germination events each year for the second experiment. Populations were very low in the third year of

each experiment (Table 2). Weed seed germination is affected by rainfall (Roberts and Potter 1980). In our study, the wet May 1996 may have caused more germination in experiment two, but germination was significantly reduced in experiment one, compared to previous year. The size of the woolly cupgrass seedbank likely had a greater effect on the population than rainfall. The largest seedbank was expected at the beginning of the first year for both experiments and the seedbank was reduced each year as no woolly cupgrass seed rain was allowed to recharge the seedbank during the experiment duration. The seedbanks in these experiments were reduced by germination events over time. Leck et al. (1989) reported that germination was one of many factors reducing the seedbank. Lower seed density in the soil might explain the fewer germination events in the second and third years of the experiments.

Woolly cupgrass populations decreased significantly after the first germination event in each year (Table 2). Tillage significantly affected the woolly cupgrass population for the second germination event in 1994 and 1995 (Table 2). Conventional tillage reduced the woolly cupgrass populations compared to no-tillage presumably by moving woolly cupgrass seeds deeper in the soil and lowering the seed population in the active seed zone.

The second germination event was actually the first germination event following the tillage treatment in each year. Significant differences in woolly cupgrass populations between no-tillage and conventional tillage were observed at this germination event, but were not evident thereafter. The fifth and sixth germination events in 1994 for the first experiment also showed significant differences which might be due to uneven and sporadic germination in the late season. In experiment two, tillage did not have significant effect on population, in part due to low initial seed density in the soil. Egley and Williams (1990) reported that

tillage during the second through the fifth year did not affect weed seed emergence due to low seed density in the soil.

There were significant differences in woolly cupgrass populations among herbicide treatments during the first two years for experiment one and in the first year for experiment two (Table 3). However, the differences among herbicide treatments were not as great as the differences between the first germination event and the rest of the germination events in the season.

At the end of the three-year experiments, the seedbank was reduced by germination and the elimination of the seed rain. Since the woolly cupgrass seedbank was depleted, there were no significant differences in soil seed density between no-tillage and conventional tillage treatments (Table 4). Further, there were no differences among herbicide treatments for experiment one (Table 5). Only three seeds were found in all soil cores sampled from the entire area and a statistical analysis of experiment two was impossible. From an agronomic perspective, woolly cupgrass seedbank was depleted at the end of the third year for experiment one, and at the end of the second year for experiment two.

Hatternman-Valenti et al. (1996) reported that woolly cupgrass seeds at physiological maturity were dormant and seeds collected from the soil surface in February were non-dormant. Obviously, woolly cupgrass seeds are ready to germinate in the spring unless the environment is not favorable for germination. Owen (1990) also reported woolly cupgrass could germinate and emerge from a depth of 9 cm in the field. Low dormancy, high viability, and the ability to germinate deep in the soil enable woolly cupgrass to have several germination flushes in one season, each potentially with a large number of seedlings.

However, a great proportion of woolly cupgrass seeds in the seedbank might germinate in the first growing season. Egley and Williams (1990) also reported that 61 to 88% of all weed seeds from a seed rain emerged during the first year. Thus the woolly cupgrass seedbank would deplete quickly with no seed rain recharging the seedbank. However, even when weed seedbanks were reduced up to 90%, poor weed control in one year could restore soil seedbank to 50% of the original level (Burnside et al. 1986). Woolly cupgrass, under uncompetitive conditions, can produce more than 160,000 seeds per plant (Owen 1990). Thus, seedbank could build up in one growing season if only a few plants escaped control.

The effects of tillage on germination depth varied for the experiments (Table 6). Conventional tillage caused the woolly cupgrass to germinate deeper in the soil. Significant differences were only observed during the second through the fourth germination events in the first year and the second germination event in the second year of the first experiment. Average germination depths of 3.8 cm in conventional tillage and 1.8 cm in no-tillage for the second germination event in 1994 were observed (Table 6). This was consistent with Yenish et al. (1992) who demonstrated that over 60% of all weed seeds in the top 19 cm of soil were found in the top 1 cm in no-tillage sites, while moldboard plowing uniformly distributed weed seeds in the top 19 cm of soil. Conventional tillage caused uniform distribution of woolly cupgrass in the tilled zone, resulting in more seeds germinating deeper. The tillage effects on germination depth were most evident for the first germination event following tillage which was the second germination event in our study. Tillage effects on germination depth did not last for the entire growing season. This might be attributed to low seedling density in the late season.

No significant difference of tillage was observed for experiment two, possibly due to large germination variation within replications. Trends suggested that the woolly cupgrass germinated deeper in the late season for years two and three in both experiments. Tillage effects on germination depth over time were significant in the first two years for both experiments. The average germination depth at the beginning of the experiments was 1.3 cm and increased to 5.2 cm at the end of the third year. Data were averaged over two experiments.

In both experiments, significant differences in seedling density among herbicide treatments were observed in the second and the third germination events of the first year and the second germination event of the second year (Table 3). In 1996, herbicides effectively controlled the second germination event in herbicide treated plots. However, no individual herbicide provided consistently better control than other herbicides. Since seedlings from the first germination event were controlled by the application of glyphosate in each year, this might mask any herbicide effects. Herbicides significantly affected germination depth of the second germination event for experiment one in 1994 (Table 7). Seeds germinated deeper in herbicide treatments when compared to the untreated control. This might be attributed to the good control of woolly cupgrass seedlings in the upper layer of the soil where preemergence herbicide concentration was presumed highest.

At late germination events of each year, there were usually significant differences in germination depth among herbicide treatments. However, the herbicide at this time might not be able to control the late germination of woolly cupgrass due to low concentration. Importantly, late season germination events were usually sporadic and variable, thus

differences observed in the later season probably were not likely responding to the herbicide effects.

In conclusion, our study showed that conventional tillage significantly decreased woolly cupgrass populations and caused woolly cupgrass to germinate deeper in one experiment but not in the other experiment. The implications of this variable response are not known, but differences were likely attributable in part to the initial woolly cupgrass population. Preemergence herbicides had variable effects on the woolly cupgrass population. Herbicides only had significant effects on germination depth for the second germination event of 1994. Herbicides eliminated seedlings germinating from the upper soil depths; this resulted in no significant difference in germination depth among herbicides in later events. When seed production was eliminated, the woolly cupgrass seedbank was depleted in three years.

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Table 1. Precipitation for the Armstrong Research Farm, Lewis, IA, during 1994 to 1997 growing seasons.

| Year | Precipitation | | | |
|---|---|---|---|---|
| | May | June | July | August |
| | mm | | | |
| 1994 | 15 (-84) | 220 (+114) | 65 (-20) | 87 (-16) |
| 1995 | 178 (+79) | 81 (-25) | 67 (-19) | 69 (-34) |
| 1996 | 245 (+146) | 192 (+86) | 78 (-8) | 67 (-36) |
| 1997 | 67 (-32) | 100 (-6) | 44 (-42) | 61 (-42) |
| 30-yr avg. | 99 | 106 | 86 | 103 |

Values in the parentheses represent deviation from 30-year average.

Table 2. Effects of tillage, years and germination events on woolly cupgrass seedling density.

| Germination event | | 1[a] | 2 | 3 | 4 | 5 | 6 |
|---|---|---|---|---|---|---|---|
| | | Seedlings $m^{-2}$ | | | | | |
| | Tillage[b] | | | | | | |
| | | **Experiment 1** | | | | | |
| 1994 | NT | 517 a[c] | 297 a | 183 a | 43 a | 151 a | 11 b |
| | CT | 484 a | 140 b | 205 a | 43 a | 65 b | 22 a |
| 1995 | NT | 695 b | 124 a | 90 a | 33 a | | |
| | CT | 884 a | 80 b | 79 a | 14 b | | |
| 1996 | NT | 207 a | 150 a | 3 a | 0 a | | |
| | CT | 143 a | 58 a | 2 a | 0 a | | |
| | | **Experiment 2** | | | | | |
| 1995 | NT | 297 a | 37 a | 45 a | 17 a | | |
| | CT | 195 a | 44 a | 30 b | 12 a | | |
| 1996 | NT | 254 a | 41 a | 1 a | 0 a | | |
| | CT | 127 b | 26 a | 1 a | 0 a | | |
| 1997 | NT | 9 a | 1 a | 1 a | 0 a | | |
| | CT | 4 b | 0 a | 0 a | 0 a | | |

[a] Data from first germination event were collected before herbicide and tillage treatments.
[b] NT: No-tillage; CT: Conventional tillage.
[c] Means within year in same column followed by the same letter are not significantly different at P=0.05, according to the LSD test.
Data are averaged over five herbicide treatments.

Table 3. Effects of preemergence herbicides, years and germination events on woolly cupgrass seedling density.

| Germination event | | | 1[a] | 2 | 3 | 4 | 5 | 6 |
|---|---|---|---|---|---|---|---|---|
| | Herbicides | kg ha$^{-1}$ | Seedlings m$^{-2}$ | | | | | |
| | | | **Experiment 1** | | | | | |
| | Acetochlor | 1.2 | 541 a[b] | 281 b | 239 a | 59 a | 124 a | 12 ab |
| | Dimethenamid | 0.7 | 451 a | 239 b | 208 ab | 43 a | 110 a | 18 a |
| 1994 | Metolachlor | 1.4 | 468 a | 228 b | 121 b | 42 a | 97 a | 15 ab |
| | Pendimethalin | 0.9 | 437 a | 216 b | 199 ab | 36 a | 87 a | 11 b |
| | Untreated control | | 599 a | 974 a | 181 ab | 31 a | 124 a | 10 b |
| | Acetochlor | 1.2 | 962 a | 50 b | 105 a | 31 a | | |
| | Dimethenamid | 0.7 | 790 ab | 108 ab | 87 a | 25 ab | | |
| 1995 | Metolachlor | 1.4 | 749 ab | 109 ab | 57 a | 11 b | | |
| | Pendimethalin | 0.9 | 767 ab | 92 ab | 69 a | 11 b | | |
| | Untreated control | | 678 b | 150 a | 104 a | 46 a | | |
| | Acetochlor | 1.2 | 184 a | 0 b | 2 a | 0 a | | |
| | Dimethenamid | 0.7 | 205 a | 0 b | 2 a | 0 a | | |
| 1996 | Metolachlor | 1.4 | 117 a | 0 b | 1 a | 0 a | | |
| | Pendimethalin | 0.9 | 138 a | 0 b | 2 a | 0 a | | |
| | Untreated control | | 229 a | 104 a | 4 a | 1 a | | |

[a] Data from first germination event were collected before herbicide and tillage treatments.
[b] Means within year in same column followed by the same letter are not significantly different at P=0.05, according to the LSD test. Data are averaged over two tillage treatments.

Table 3. Effects of preemergence herbicides, years and germination events on woolly cupgrass seedling density. (cont.)

| Germination event | | | 1[a] | 2 | 3 | 4 |
|---|---|---|---|---|---|---|
| | Herbicides | kg ha$^{-1}$ | Seedlings m$^{-2}$ | | | |
| | | | **Experiment 2** | | | |
| | Acetochlor | 1.2 | -[c] | 26 b[b] | 24 b | 16 a |
| | Dimethenamid | 0.7 | - | 38 ab | 42 b | 14 a |
| 1995 | Metolachlor | 1.4 | - | 36 ab | 23 b | 14 a |
| | Pendimethalin | 0.9 | - | 42 ab | 25 b | 13 a |
| | Untreated control | | 246 | 59 a | 72 a | 14 a |
| | Acetochlor | 1.2 | 209 a | 0 b | 1 a | 0 a |
| 1996 | Dimethenamid | 0.7 | 187 a | 0 b | 1 a | 0 a |
| | Metolachlor | 1.4 | 244 a | 0 b | 1 a | 0 a |
| | Pendimethalin | 0.9 | 153 a | 0 b | 1 a | 0 a |
| | Untreated control | | 159 a | 34 a | 1 a | 0 a |
| | Acetochlor | 1.2 | 7 a | 0 a | 1 a | 0 a |
| | Dimethenamid | 0.7 | 8 a | 0 a | 0 a | 0 a |
| 1997 | Metolachlor | 1.4 | 7 a | 0 a | 1 a | 0 a |
| | Pendimethalin | 0.9 | 8 a | 1 a | 0 a | 0 a |
| | Untreated control | | 5 a | 1 a | 0 a | 0 a |

[a] Data from first germination event were collected before herbicide and tillage treatments.
[b] Means within year in same column followed by the same letter are not significantly different at P=0.05, according to the LSD test. Data are averaged over two tillage treatments.
[c] Only data from untreated control plot were collected.

Table 4. Effect of tillage on woolly cupgrass seedbank.

| | Experiment 1 | Experiment 2[b] |
|---|---|---|
| | Seeds $m^{-2}$ | |
| No-tillage | 97 a[a] | 14 |
| Conventional-tillage | 42 a | 7 |

[a] Means within a column followed by the same letter are not significantly different at P=0.05, according to the LSD test. Data are averaged over five herbicide treatments.
[b] Statistical analysis for experiment 2 was not possible due to no seeds recovered from many plots.
Samples were taken after the conclusion of a three year experiment.

Table 5. Effects of preemergence herbicides on woolly cupgrass seedbank.

| | Experiment 1 | Experiment 2[b] |
|---|---|---|
| | Seeds $m^{-2}$ | |
| Acetochlor | 70 a[a] | 0 |
| Dimethenamid | 70 a | 18 |
| Metolachlor | 18 a | 0 |
| Pendimethalin | 104 a | 34 |
| Untreated control | 86 a | 0 |

[a] Means within a column followed by the same letter are not significantly different at P=0.05, according to the LSD test. Data are averaged over two tillage treatments.
[b] Statistical analysis for experiment 2 was not possible due to no seeds recovered from many plots.
Samples were taken after the conclusion of a three year experiment.

Table 6. Effects of tillage, years and germination events on woolly cupgrass germination depth.

| Germination event | Tillage | Germination depth | | | | | |
|---|---|---|---|---|---|---|---|
| | | 1[a] | 2 | 3 | 4 | 5 | 6 |
| | | cm | | | | | |
| | | **Experiment 1** | | | | | |
| 1994 | NT[c] | 1.0 a[b] | 1.8 b | 1.1 b | 1.7 b | 1.8 a | 1.0 a |
| | CT | 1.2 a | 3.8 a | 2.2 a | 3.0 a | 2.0 a | 1.8 a |
| 1995 | NT | 2.5 a | 2.2 b | 3.0 a | 3.4 b | | |
| | CT | 2.4 a | 2.7 a | 3.5 a | 4.6 a | | |
| 1996 | NT | 4.4 a | 3.7 a | 5.0 a | -[d] | | |
| | CT | 4.7 a | 3.1 a | 5.2 a | - | | |
| | | **Experiment 2** | | | | | |
| 1995 | NT | 1.6 a | 1.8 a | 2.6 a | 3.2 b | | |
| | CT | 1.5 a | 1.9 a | 2.5 a | 4.2 a | | |
| 1996 | NT | 3.7 a | 5.2 a | 6.0 a | - | | |
| | CT | 4.1 a | 3.4 a | 5.4 a | - | | |
| 1997 | NT | 5.3 a | - | - | - | | |
| | CT | 5.3 a | - | - | - | | |

[a] Data of first germination event were collected before herbicide and tillage treatments.
[b] Means within year in same column followed by the same letter are not significantly different at P=0.05, according to the LSD test. Data are averaged over five herbicide treatments.
[c] NT: No-tillage; CT: Conventional tillage.
[d] Not enough seedlings to determine germination depth.

Table 7. Effects of herbicides, years and germination events on woolly cupgrass germination depth.

| Germination event | | kg ha$^{-1}$ | Germination depth | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | 1[a] | 2 | 3 | 4 | 5 | 6 |
| | Herbicides | | cm | | | | | |
| | | | **Experiment 1** | | | | | |
| | Acetochlor | 1.2 | 1.2 a[b] | 2.5 bc | 1.8 a | 1.7 c | 1.8 a | 1.3 a |
| | Dimethenamid | 0.7 | 1.2 a | 2.4 bc | 1.5 a | 1.8 bc | 2.2 a | 1.3 a |
| 1994 | Metolachlor | 1.4 | 1.2 a | 3.0 ab | 1.7 a | 2.8 ab | 1.5 a | 1.3 a |
| | Pendimethalin | 0.9 | 1.0 ab | 3.2 a | 1.7 a | 2.2 bc | 2.0 a | 1.9 a |
| | Untreated control | | 0.9 b | 2.2 c | 1.4 a | 2.9 a | 1.9 a | 1.0 a |
| | Acetochlor | 1.2 | 2.6 ab | 2.5 a | 3.3 a | 4.0 ab | | |
| | Dimethenamid | 0.7 | 2.7 a | 2.4 a | 3.3 a | 4.1 ab | | |
| 1995 | Metolachlor | 1.4 | 2.3 ab | 2.6 a | 3.2 a | 4.3 a | | |
| | Pendimethalin | 0.9 | 2.4 ab | 2.4 a | 3.5 a | 3.9 ab | | |
| | Untreated control | | 2.2 b | 2.4 a | 2.8 a | 3.7 b | | |
| | Acetochlor | 1.2 | 4.4 a | -[c] | 5.7 a | - | | |
| 1996 | Dimethenamid | 0.7 | 4.8 a | - | 4.5 a | - | | |
| | Metolachlor | 1.4 | 4.7 a | - | 5.3 a | - | | |
| | Pendimethalin | 0.9 | 4.6 a | - | 5.2 a | - | | |
| | Untreated control | | 4.4 a | 3.4 | 4.7 a | - | | |
| | | | **Experiment 2** | | | | | |
| | Acetochlor | 1.2 | -[d] | 1.8 a | 2.0 c | 3.9 a | | |
| | Dimethenamid | 0.7 | - | 1.8 a | 2.9 ab | 3.3 b | | |
| 1995 | Metolachlor | 1.4 | - | 1.7 a | 2.7 ab | 3.8 ab | | |
| | Pendimethalin | 0.9 | - | 2.2 a | 3.2 a | 3.9 a | | |
| | Untreated control | | 1.6 | 1.8 a | 2.1 bc | 3.6 ab | | |
| | Acetochlor | 1.2 | 3.8 a | - | 6.0 a | - | | |
| 1996 | Dimethenamid | 0.7 | 3.5 a | - | 5.5 ab | - | | |
| | Metolachlor | 1.4 | 4.1 a | - | 6.6 a | - | | |
| | Pendimethalin | 0.9 | 4.1 a | - | 6.1 a | - | | |
| | Untreated control | | 3.9 a | 4.3 | 4.5 b | - | | |

[a] Data of first germination event were collected before herbicide and tillage treatments.
[b] Means within year in same column followed by the same letter are not significantly different at P=0.05, according to the LSD test. Data are averaged over two tillage treatments.
[c] Not enough seedlings for determining germination depth.
[d] Only data from control plot was collected.

## GENERAL CONCLUSIONS

The mechanisms by which woolly cupgrass survived nicosulfuron application were related to tillering ability, nicosulfuron rate, and the physical constraint imposed by the leaf sheath.

Woolly cupgrass was able to produce many tillers in a short period of time. Numerous tiller buds existed at the stage that was recommended for control by nicosulfuron.

The vascular connections between the main stem and the tillers were examined. No evidence was observed that suggested a physical barrier existed between the main stem and tillers thus preventing herbicide translocation from the main stem. Autoradiographic studies demonstrated that $^{14}$C-nicosulfuron was translocated to every tiller bud regardless of herbicide placement on the plant. This evidence ruled out the possibility that the tiller regrowth was due to poor herbicide translocation to the tillers.

Seed reserves affected woolly cupgrass tiller production. However, woolly cupgrass seed reserves were used up before the first tiller emerged. It was not likely that seed reserves supported tiller growth.

Nicosulfuron inhibited the growth of the main stem. However, tillers still grew inside the leaf sheath. Tiller growth was affected by nicosulfuron rate and restricted by the leaf sheath. The recommended nicosulfuron rate usually did not inhibit tiller growth. The constraint imposed by the leaf sheath occasionally was lost and tiller buds elongated and emerged. Tiller growth following nicosulfuron application was affected by tillering ability, rate of nicosulfuron, and the loss of constraint imposed by the leaf sheath.

Conventional tillage significantly decreased woolly cupgrass populations. Germination depth of woolly cupgrass in conventional tillage was significantly deeper than in no-tillage. However, the results varied at different locations and years. There were differences in populations among herbicide treatments, however, these differences were not as important as the differences between the first germination event and subsequent germination events. Germination depth increased for later germination events, however, differences among herbicides were not significant. When seed production was eliminated, the woolly cupgrass seedbank was depleted in three years.

Further work should be done to quantify nicosulfuron distribution between the main shoot apex and the tiller buds, and to determine the lethal dose for tiller buds. Further work should be done to quantify the physical constraint of leaf sheath and determine the interaction of physical constraint and environmental factors on tiller emergence.

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# IMAGE EVALUATION
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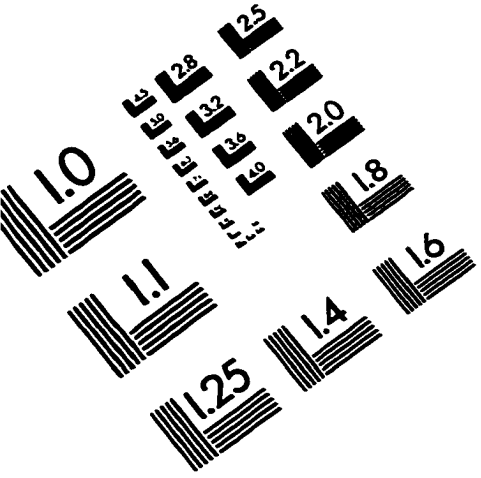


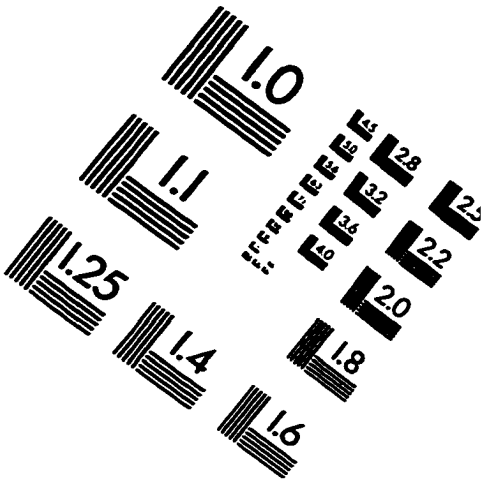


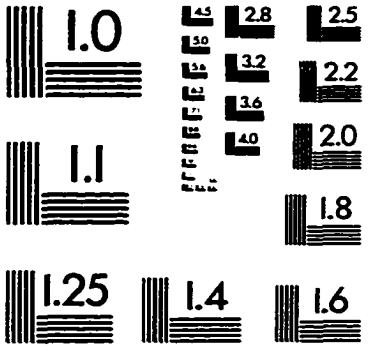


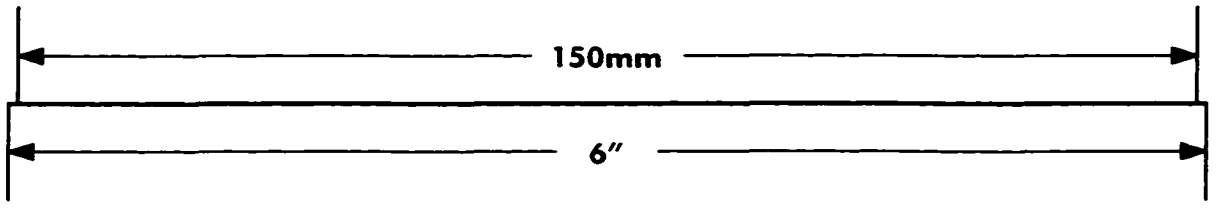


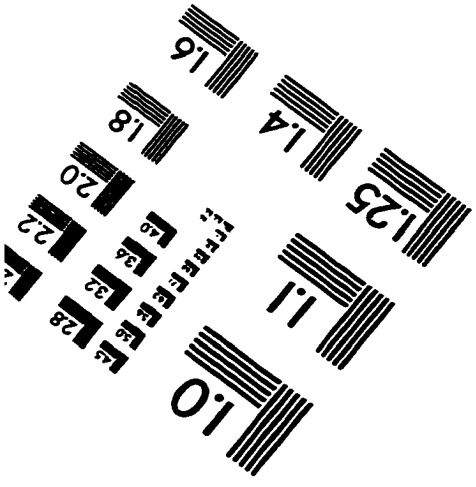


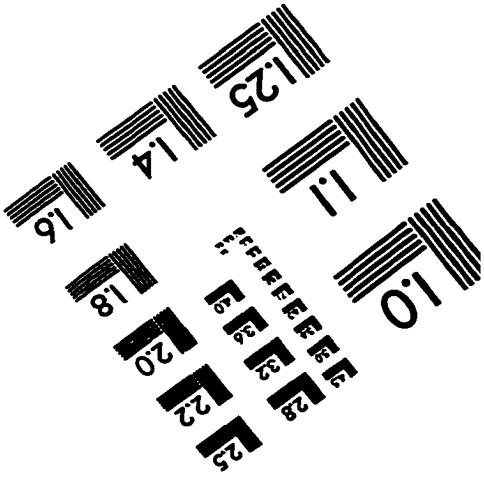


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